The Dissertation Committee for Karin Lise Akre Certifies that this is the approved version of the following dissertation:

The Cognitive Biology of Mate Choice in Túngara Frogs (*Physalaemus pustulosus*)

Committee:

Michael J. Ryan, Supervisor

Molly E. Cummings

Michael Domjan

Lee A. Fuiman

Ulrich G. Mueller

The Cognitive Biology of Mate Choice in Túngara Frogs (*Physalaemus pustulosus)*

by

Karin Lise Akre, B. A.

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Dedication

To Fisher Lake

To Pipeline Road

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The Cognitive Biology of Mate Choice
in Túngara Frogs (*Physalaemus pustulosus*)

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Karin Lise Akre, Ph.D.
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Supervisor: Michael J. Ryan

Sexual selection is responsible for a great diversity of elaborate male traits. A general female preference for males that have exaggerated traits drives this process, but the reasons females exhibit this preference are often unclear. Recent advances in understanding signal evolution have emerged from studies of receiver psychology that focus on how receivers perceive and process communication signals. I apply the perspective of receiver psychology to understand female preference for elaborate signals in túngara frogs (*Physalaemus pustulosus*).

Male túngara frogs produce advertisement calls of variable complexity. Females exhibit a strong preference for complex to simple calls, but previous studies have not found consistent patterns of preference between calls of variable complexity. In my doctoral research, I investigate the function of variable complexity in túngara frogs. Specifically, I address the following questions: 1) Are calls of variable complexity especially relevant to females in certain contexts? Do males respond to female behavior by increasing their production of complex calls? 2) Does male to female proximity

influence female response to call complexity? 3) Are females constrained by their perceptual biology in discriminating differences in call complexity? 4) Can females remember attractive males over silences between bouts of advertising? Is working memory for attractive males dependent upon signal complexity? And 5) Does signal memorability increase with signal complexity in a linear relationship?

These studies provide several new perspectives to an understanding of female preference for elaborate signals. Phonotaxis experiments demonstrate that females use elicitation behaviors to influence male production of complex calls, that proximity influences female response to signal elaboration, that females are constrained by their perceptual biology in discriminating between complex calls, that memory can influence the evolution of signal complexity, and that memorability and signal complexity share a non-linear relationship.

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Introduction

Sexual selection is responsible for a great diversity of elaborate male traits (Andersson 1994; Darwin 1871). A general female preference for males that have exaggerated traits drives this process (Ryan & Keddy-Hector 1992), but the reasons females exhibit this preference are often unclear. The evolution of female preferences can occur through a variety of both direct and indirect selection processes (Kirkpatrick & Ryan 1991), but this does not fully explain why preferences evolve in the direction that favors elaboration. This question has been addressed through both ultimate and proximate perspectives on how signal elaboration influences receiver response (Guilford & Dawkins 1991; Hebets & Papaj 2005). In some cases, signal elaboration can serve a content-related function, such indicating the signaler's quality (McGraw & Hill 2000). Signal elaboration can also increase sensory stimulation and influence cognitive processing in ways that bias female choice, such as releasing females from habituation to repeated signals (Hartshorne 1973; Searcy 1992; Stripling et al. 1997). Thus a number of reasons can contribute to explaining the widespread female preference for elaborate traits, yet in many cases the relationship between female preference and male trait elaboration remains unclear.

Understanding female response to elaborate signals can be complicated by variability in female response (Jennions & Petrie 1997). Within individuals, preference varies according to both extrinsic and intrinsic factors, such as the risk of predation or parasitism, previous experience, and cyclical changes in hormonal state (Bertram et al. 2004; Hebets 2003; Jennions & Petrie 1997; Lynch et al. 2006; Ryan et al. 2007; Wagner et al. 2001). The relationship between female response and male trait variation can also take many forms that all impose directional selection on traits to evolve increased

elaboration (Lande 1981; Ritchie et al. 2001; Wagner 1998). Some females respond to increasing trait values with a linear increase in preference (Basolo 1998), but in many cases female response to increasing trait values instead either peaks at an intermediate value or increases along a logarithmic curve (Cohen 1984; Lande 1981; Ryan & Rand 1993; Wagner 1998). The reasons for variation in this relationship are even less understood than the general preference for male trait exaggeration.

Recent advances in understanding signal evolution have emerged from studies of receiver psychology, which focus on how receivers perceive and process communication signals (Dukas 2004; Guilford & Dawkins 1991; Rowe 1999; Rowe & Skelhorn 2004). This area of research demonstrates that cognitive abilities of receivers, such as attention, learning, and memory, can influence the evolution of communication signals, and many of these factors remain unexplored in the domain of mating communication (Ryan et al. 2009). Pursuing the perspective of receiver psychology further might illuminate more reasons why females prefer elaborate signals, and might also explain why patterns of female response to increasing trait values show such variability (Ryan 1990).

I investigate female response to variable signal complexity in túngara frogs (*Physalaemus pustulosus*). Male túngara frogs produce advertisement calls that can have two parts: a low frequency sweep called a "whine," and a high frequency burst called a "chuck" (Ryan 1985; Ryan & Rand 2003a). Whines may be voiced alone (simple calls), or followed by 1 to 7 chucks (complex calls). Females have clearly promoted the evolution of túngara frog signal complexity, because they exhibit a strong preference for complex over simple calls (Gridi-Papp et al. 2006). The role of female preference in driving the production of multiple chucks is predicted by sexual selection theory, yet previous studies have not found consistent patterns of preference for greater complexity, or more over fewer chucks (Bernal et al. 2009). A lack of preference for greater

complexity is especially intriguing because frog-eating bats preferentially approach males that produce chucks (Ryan et al. 1982), and preferentially approach males that produce more chucks over males that produce fewer chucks (R. A. Page, pers. comm.). Multiple chucks should provide some benefit to males that balances the predation risk associated with these calls. Thus the lack of consistent female preference for greater complexity has called into question the function of multiple chucks. I addressed this question from the perspective of receiver psychology, examining a number of factors that introduce variability into female response to mating signals.

Males that produce dynamic mate attraction signals can often use their signals strategically, and show sensitivity to environmental variables that shift the optimum signal value at any given moment (Lindstrom et al. 2009). Túngara frogs rarely produce more than 2 chucks in natural settings (Bernal et al. 2007), presumably due to the risk of frog-eating bats. They increase chuck production, however, in response to male competition (Bernal et al. 2009; Goutte et al. 2010). Although observations have suggested that males increase chucks in response to female approach (S. Rand & M. Ryan, pers. comm.), no studies have quantified this behavior. If males respond to females by increasing chuck production, this could define a context in which chuck number is relevant to females. In chapter 1, I describe female behavior when approaching males in a naturalistic low-density chorus and define two types of movements: those that are used for mate acquisition, and those that are not. I present a quantitative analysis of male response to natural variation in female behavior in order to statistically test the hypothesis that females perform a repertoire of non-approach behaviors that function to elicit more chucks from calling males. The analysis in chapter 1 demonstrates that males increase chuck number in response to female movements, and

female elicitation behaviors influence the probability that males will transition to producing more chucks.

In chapter 2, I conduct controlled laboratory experiments to determine whether proximity is a context that influences female response to multiple chucks, as suggested by the analysis in chapter 1. Male to female proximity is an important contextual factor in mating communication for diverse taxa (How et al. 2008; Patricelli & Krakauer 2010). Proximity-dependent response to acoustic signals, although known to occur in birds, anurans, and insects (Beckers & Schul 2004; Doolan & Gerald 1985; Marquez et al. 2008; Naguib & Wiley 2001), is somewhat surprising, because acoustic signals have a large active space, that is, they are detected over a great distance. The discrimination experiments in chapter 2 demonstrate that female túngara frogs do prefer greater complexity when exposed to high amplitude signals that indicate close proximity to males, but not when exposed to lower amplitude signals that indicate greater distance from males. The reason for proximity-dependent response is not clear, since it could reflect an inability to discriminate low amplitude calls, or a lack of motivation to respond to signal differences that they are capable of discerning.

In chapter 3, I examine the close-proximity preference for greater complexity in more detail. I ask whether females exhibit a general preference for greater complexity, and demonstrate that even in close proximity to males, females show diminishing response to increasing chuck number. Females sometimes exhibit diminishing response to male traits beyond an intermediate value when attraction to extreme values would risk heterospecific mating (Pryke & Andersson, 2008), but this possibility is not relevant for túngara frogs. Several theoretical studies predict that diminishing female response to increasing elaboration could result from constraints of female perceptual biology such as those predicted by Weber's law (Cohen 1984; Jennions & Petrie 1997; Ryan 1990).

Weber's law is a psychophysical model of perception that predicts the threshold change required to notice a difference in stimulus magnitude (Yost 1994). The analysis in chapter 3 demonstrates that female response significantly matches the predictions of Weber's law. This provides strong evidence that female ability to discriminate is constrained by their perceptual biology.

Another aspect of receiver psychology that can interact with stimulus complexity is memory, which has been explored in the evolution of aposematic signals, but few other domains of animal communication (Fetterman 1996; Rowe 1999; Speed 2000). In chapter 4, I examine the role of memory in the evolution of mating signal complexity. Female túngara frogs assess males in choruses that call in bouts separated by silent intervals, and retaining attraction to a male through these silent periods could benefit females by reducing the time spent assessing potential mates. I conducted modified phonotaxis tests to determine whether females retain attraction to complex calls over variable silent periods in a delayed choice experiment. Females do retain attraction to calls with 3 chucks, but not calls with 1 chuck. I analyze whether this result relates to differences in motivation or postural orientation towards attractive stimuli, and conclude that the differential response results from differential memory. This result highlights the role of receiver psychology in signal evolution, and introduces a previously unreported reason that females mate with males that produce more elaborate signals.

In chapter 5, I further explore the relationship between signal complexity and memory, testing whether signal memorability increases linearly with complexity. This analysis first demonstrates that the relationship between memorability and signal complexity is non-linear. I then explore several factors that might explain this non-linear relationship, including the duration of the chuck series, the number of chucks, and the temporal relationship between call components. These experiments suggest that

backward masking, a process by which the presence of a later stimulus component effectively masks an earlier one, might play a role in reducing the memorability of highly complex stimuli.

These studies provide several new perspectives to an understanding of female preference for elaborate signals: the use of elicitation behaviors, the importance of proximity in defining the context for signal response, an adherence to Weber's law constraining preference, and the role of female memory in the evolution of mating signals. These results highlight the importance of appreciating variability in female preference response and they demonstrate the role of receiver psychology in the evolution of mating signals.

Chapter 1: Females elicit riskier mating signals

ABSTRACT

Sexual selection is responsible for the evolution of costly elaborate male traits. When male displays are dynamic, display strategy is sensitive to contextual cues that alter the relative costs and benefits of producing each signal. Since females often prefer more elaborate signals, males often respond to female presence by elaborating their display. When added elaboration increases assessment information or reproductive stimulation, females might benefit by extracting the maximum amount of signal elaboration from males. Thus we expect that females could exaggerate their presence and cause males to produce even costlier signals by exhibiting "elicitation" behaviors. We asked whether female túngara frogs elicit increased call complexity from prospective mates, which increases both attractiveness and predation risk. We found that females exhibit a repertoire of movements that function not in mate acquisition, per se, but in display manipulation, by eliciting increased complexity. Thus females actively influence males to produce riskier signals.

INTRODUCTION

Female choice can cause the evolution of elaborate male traits through sexual selection in spite of the costs to elaboration imposed by natural selection (Andersson 1994; Darwin 1871; Kirkpatrick & Ryan 1991; Zuk & Kolluru 1998). The distribution of sexually selected male traits is often envisioned with a peak between two divergent optimum values imposed by sexual and natural selection (Maynard Smith 1982). The distinction between static and dynamic male traits complicates this scenario. Males that produce dynamic mate attraction signals can use signals strategically, and are sensitive to

environmental variables that shift the optimum signal value at any given moment (Lindstrom et al. 2009). Contextual factors such as predation risk can influence a display strategy to shift towards the natural selection optimum (Bertram et al. 2004; Magnhagen 1991), while male competition (Goutte et al. 2010; Wong & Cando 2005) or female presence (Gautier et al. 2008) can influence a strategy to shift towards the sexual selection optimum. Many males adjust signaling (How et al. 2008) and competitive behavior (Jackson et al. 2006) according to a female's proximity. Producing energetically costly but attractive signals according to female proximity can maximize male fitness (Patricelli & Krakauer 2010). Males can even allocate energetic signaling investment according to female quality (Wong & Svensson 2009).

Displaying males respond not only to female proximity but also to female behaviors. Males can increase fitness by responding to female behaviors that indicate the likelihood of mating. For example, males can reduce energy and time costs if they avoid courting unreceptive females, or they can increase the chance of mating by producing the signals that are most attractive to females. Males commonly respond to female proceptive behavior that regulates the progression of courtship (Balsby & Dabelsteen 2002; Crews 2002), and specifically to signals that communicate receptivity (Watkins 1997) or over-stimulation (Patricelli et al. 2004). Female signaling can also influence song repertoire development (West & King 1988), and female resistance behavior can incite male competition (Cox & Le Boeuf 1977). Changes in female behavior can also influence male use of mating coercion (Evans et al. 2002).

Given the robust occurrence of male response to female behavior prior to mating, we predict that females should evolve suites of behaviors that function in eliciting from males displays that lie closer to the sexual selection optimum signal value, in spite of the increasing costs (Figure 1.1). When increasingly elaborate traits provide more

assessment information or reproductive stimulation, females might benefit by extracting the maximum amount of signal elaboration from a potential mate. Such expectations are generally consistent with predictions of theories as disparate as the Handicap Principle (Zahavi 1975; Zahavi & Zahavi 1997), chase-away sexual selection (Arnqvist & Rowe 2005; Holland & Rice 1988), and the influence of sexual stimulation on reproductive physiology (Adkins-Regan 2005; Lehrman 1965). We refer to behaviors that function to elicit from males riskier signals as "elicitation" behaviors. Elicitation behaviors are related to proceptive behaviors that solicit or maintain sexual interactions (Beach 1976), but function specifically to cause a potential sexual partner to increase its sexual display intensity. The ability to manipulate the male trait distribution towards the sexual selection optimum would demonstrate a female role in mating behavior that has not been previously established.

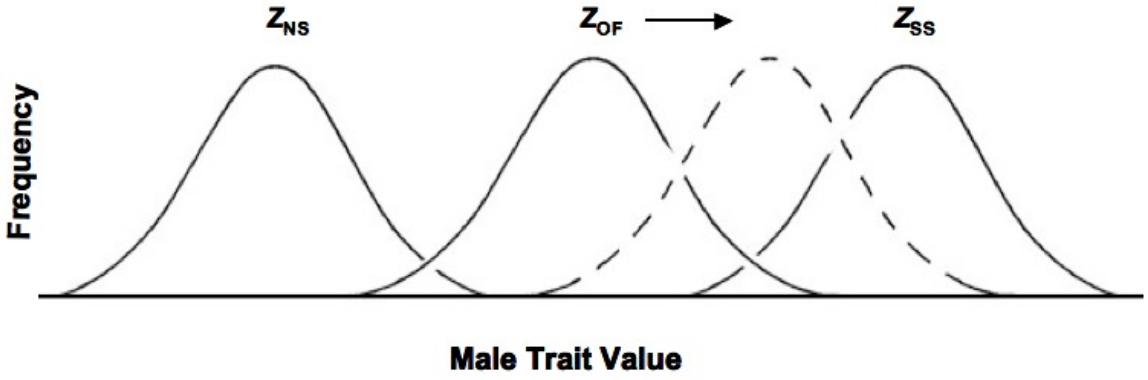


Figure 1.1: Optimal Male Trait Values

Females could influence male behavior such that he produces signals closer to the sexual selection optimum value. Solid curves shows optimal trait values under natural selection (Z_{NS}), sexual selection (Z_{SS}), and actual trait values at the average, as overall fitness (Z_{OF}). Dashed curve shows how actual trait values might shift under the influence of females eliciting signal elaboration.

Although females might share some costs of elaborate male signaling, the costs are borne primarily by males. In some cases predators are attracted to elaborate signals due to their enhanced conspicuousness (Endler 1978; Zuk & Kolluru 1998), and although both males and females are sensitive to predation risk during conspicuous courtship (Bertram et al. 2004; Booksmythe et al. 2008; Dill et al. 1999), the risk is expected to be higher for males because they produce conspicuous signals. Elaborate signals may also be costly due to their energetic investment (Patricelli & Krakauer 2010), and in these cases females are likely to bear none of the cost of elaborate signals. Thus if females prefer more elaborate signals, the sexual selection optimum will always differ from both the natural selection optimum and the overall fitness optimum signal value, and males might resist producing the sexual selection optimum.

We asked whether females perform elicitation behaviors that actively influence males to increase production of costly mating signals in a study focusing on túngara frogs (*Physalaemus pustulosus*), a species with mating signals of established variable costs.

Túngara Frog Natural History

Male túngara frogs use advertisement calls to attract mates. The advertisement calls can have two components: an initial "whine," and a terminal "chuck" (Ryan 1985; Ryan & Rand 2003a). Whines can be voiced alone (simple calls), or followed by one to seven chucks (complex calls). We define signal complexity as the number of chucks affixed to the whine (Akre & Ryan 2010; Ryan 1985; Ryan & Rand 1981). Females show a robust preference for complex to simple calls (Gridi-Papp et al. 2006) and a context-dependent preference for greater complexity [calls with more chucks to calls with fewer chucks (K. L. Akre & M. J. Ryan, in prep)]. Males call in choruses, and within a single call bout one male may produce variable call types, including both simple and

complex calls. When females choose a male, they pair in amplexus and then leave the chorus site for a period of up to a few hours before finding a place to release and fertilize eggs.

Túngara frog males are exposed to predation risk from frog-eating bats that are common at chorus sites (Ryan et al. 1982). These predators preferentially attack males producing complex calls (Ryan et al. 1982), and prefer calls of greater complexity (R. A. Page, unpublished data). Thus males face a conflict whereby they will improve reproductive success by producing calls with more chucks but increase the probability of mortality with those same calls.

When females choose a mate, they appear to sample several males in a chorus (Ryan 1985) and then move directly towards a male to form amplexus (Figure 1.2). Observations of females in small choruses show that in addition to this standard choice behavior, females exhibit a repertoire of ancillary behaviors that do not function to directly approach or retreat from a male, but instead seem to capture a male's attention and elicit increased behavioral response from him (Figure 1.2). We tested whether these ancillary behaviors elicit increased signal elaboration from males.

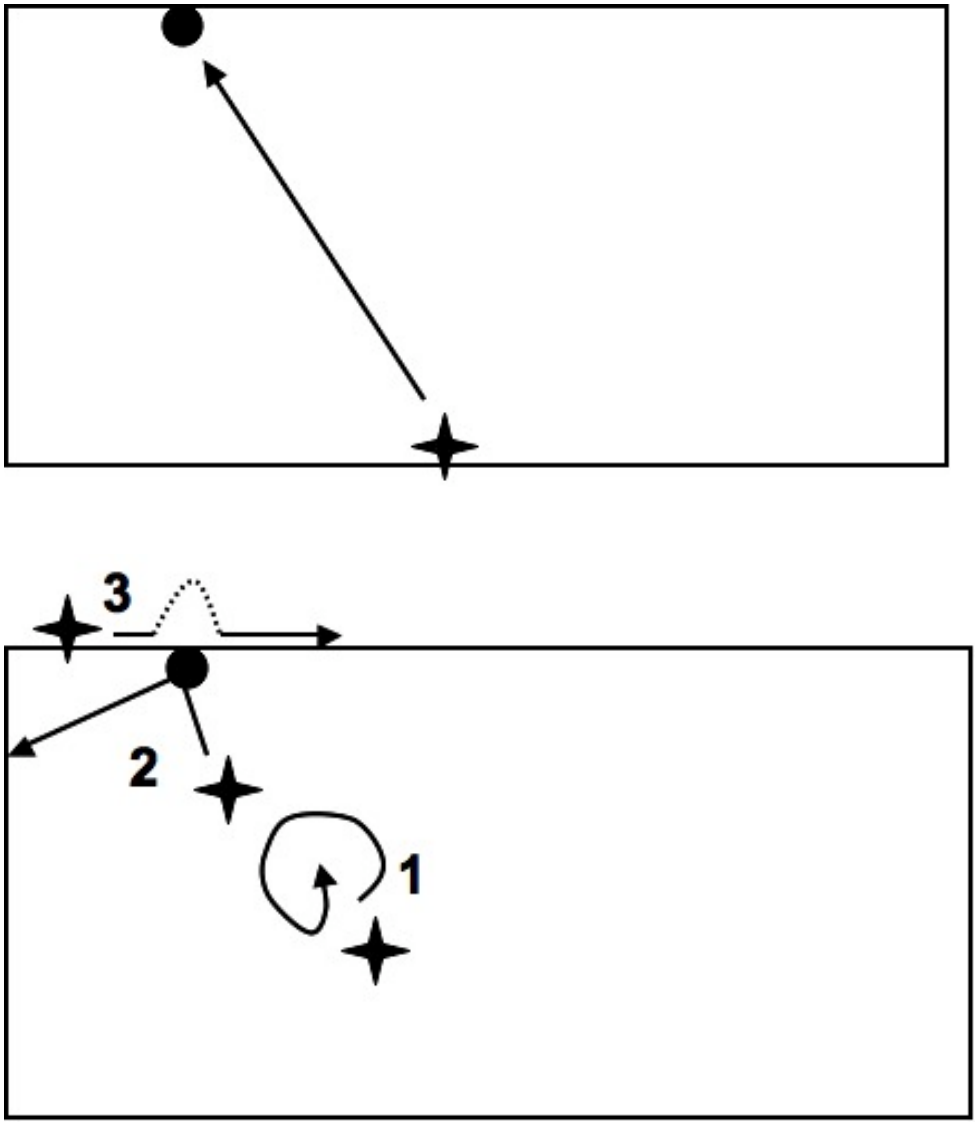


Figure 1.2: Female Locomotive Behaviors

Females exhibit a repertoire of locomotive behaviors. Above are drawings of frog movement at the breeding pond, as in Ryan 1985 (female = star, male = circle, path = arrowed lines). Immediately prior to amplexus, a female approaches a male with directed movements (upper panel). Females sometimes also produce "not approach–retreat" movements, such as those shown above (lower panel). These numbered movements represent (1) Water Circle; (2) Body Bump; and (3) Jump Over.

METHODS

Data were collected in Gamboa, Panama at the Smithsonian Tropical Research Institute (STRI) between June and September of 2008. We captured single calling males and male-female pairs in amplexus. We tested couples in a 170 x 70 cm cement pond enclosed in a 184 x 92 cm PVC and plastic tarp fence in a clearing adjacent to rainforest. We released all frogs after testing at the site of collection, either in pairs or single, as they were found. We used each frog once, and we followed standard toe-clipping procedures to mark individuals before release. Toe clip procedures followed the Guidelines for Use of Live Amphibians and Reptiles in Field and Laboratory Research, compiled by the Herpetological Animal Care and Use Committee (HACC) of the American Society of Ichthyologists and Herpetologists, available at: http://www.asih.org/files/hacc-final.pdf. All experiments were licensed and approved by STRI, The University of Texas at Austin IACUC, and La Autoridad Nacional del Ambiente.

We used a SONY DCR-SR45 Handycam Camcorder with night vision illumination to make video and audio recordings of males responding to female behavior prior to forming amplexus. We began each trial by releasing a male into the enclosed pond and allowing him to acclimate and begin calling, either naturally or in response to mimic vocalizations. If he did not begin calling within 10 min, we released a second male. We continued to add males until at least one male called consistently. The number of males in the enclosure during a trial varied from 1 to 4. When at least one male was calling, we released a female into the enclosure on a patch of land that was obstructed from the males' view by a PVC pipe placed between the female and the water where males called. Once the female was released we began recording the trial, and continued until the female formed amplexus with a male. The female was then reunited with her

original male in a plastic container. We used JWatcher™ Video V1.0 to mark the temporal sequence of all female movements and male calls.

We asked if females actively influence male call production with their ancillary behaviors. We predicted that if females do use these behaviors to elicit greater call complexity, these movements should be different from the behaviors used to approach or retreat from a male, and followed by an immediate increase in number of chucks produced by the calling male. We also predicted that the number of these movements produced by a female should be (a) negatively correlated with the greatest number of chucks produced prior to female movement, because there is less need for eliciting chucks as the number of chucks already produced increases; (b) negatively correlated with chorus size, because larger choruses produce more chucks (Bernal et al. 2007); and (c) positively correlated with the increase in chuck number that occurs between first female movement and amplexus. These correlations should not occur for mate acquisition movements used to approach or retreat from males. We also predicted that if increasing chuck number is costly, the probability of increasing chucks in response to females should negatively correlate with the number of chucks already produced.

Defining Behaviors

We observed all female movements and defined a set of repeatable and recognizable locomotive behaviors. We determined whether or not each behavior clearly moved the female either closer to or farther from the male. If so, we labeled the behavior as approach/retreat (AR), and if not, we labeled the behavior as non-approach/retreat (NAR). If NAR behaviors elicit greater number of chucks from males we consider them elicitation behaviors because they do not appear to serve the function of mate acquisition, but instead appear to influence male display. If NAR behaviors function to influence

male display, they should result in greater escalation of chuck number than is caused by AR behaviors. If AR behaviors result in a lesser escalation of chuck number, they are not considered elicitation behaviors because the primary function is mate acquisition; the escalation of the male's display is an incidental consequence of that movement.

To score videos for female behaviors, we first familiarized ourselves to recognize the behaviors consistently and then scored all videos with the acoustic track playing to ensure the temporal sequence of events. A naive person then randomly selected 25% of the trials (10 trials) for scoring female behaviors in the absence of sound to ensure that our scoring was not biased by the males' calls.

Male Response

We tested whether female movements influence the probability that males decrease, maintain, or increase the number of chucks they produce. We predicted that a) males would be more likely to increase chuck number when females produced a movement than when females were still, and b) NAR movements would be more likely to increase chuck number than AR movements. To test these predictions, we first conducted Markov chain analysis using UNCERT (Suggs & Simmons 2005) to test whether call sequences occur as a first order semi-Markovian process, then used this to justify considering each male's call bout as a series of dyadic call transitions (an initial call and the following call). We tested whether female movement influenced the probability that following calls decreased, maintained, or increased in chuck number relative to the initial call.

We determined the first female movement that males could perceive to be the first movement after crossing the PVC pipe barrier. Females do not vocalize, so males could use visual or tactile water vibration cues to detect female presence. Our estimate of a

male's perception overestimates when he might have first detected her (i.e. he could not see her or detect her surface wave vibrations before crossing the barrier, but he might have detected her later). This would bias our results toward showing no effect of female movement on male calling.

We used the Generalized Estimating Equations (GEE) procedure in the GLM function of SPSS 16 to create a linear model of male response probabilities. Our model included the following independent variables: initial chuck number, transition type (decrease, maintain, or increase), and female movement (absent or present, and AR or NAR). The dependent variable was the male's response probability. Thus each individual had several data points in the model, and we used the repeated-subject function to control for individual male differences. We used the EM Means procedure with a sequential Bonferroni correction to generate pair-wise comparisons.

Number of Female Movements

We analyzed correlations between number of female movements and three chorus variables. To conduct these correlations we considered the subset of trials where males produced at least 1 chuck prior to the first female movement. We used this subset of data because males that don't produce chucks might be responding to current increased levels of predation risk. Males reduce chuck production in response both to approaching bats (Ryan et al. 1982) and to 'public information' that might indicate predation risk (Phelps et al. 2007). A lack of chucks could cue females to reduce conspicuous behaviors, or females themselves could perceive bat presence or public information about predation risk and reduce conspicuous behaviors accordingly.

We tested whether chorus size negatively correlated with number of female movements. We predicted that females would produce more NAR (but not AR)

movements when assessing smaller choruses because larger choruses might decrease the benefits of conspicuous movement. Males in larger choruses produce more chucks (Bernal et al. 2007), so females would have less need to elicit chucks in large choruses.

We also tested whether the number of female movements negatively correlated with the highest chuck number produced prior to her first movement. We predicted that if females use movements to elicit chucks, females would produce more NAR movements when fewer chucks were produced prior to movement, but AR movements would not differ.

We also tested whether the number of female movements positively correlated with the increase in chuck number that occurs between the first female movement and amplexus. We predicted that if females use NAR movements to increase chuck number, NAR (but not AR) movements would correlate with chuck number increase.

Complexity of Initial Call

We tested whether the complexity of the initial call influenced transition response following female movement. Since producing more chucks increases the cost of calling, we predicted a negative correlation between probability of increase and initial chuck number.

RESULTS

Defining Behaviors

Females produced 12 recognizable and repeated locomotive behaviors. Five of these behaviors were categorized as AR and 7 as NAR (Table 1.1). In acoustically-blind scoring of female movements in the 10 randomly selected trials, 69 of 73 (94.5%) of movements matched the initial, full temporal sequence scoring as AR or NAR behaviors.

Table 1.1: Classification of Female Locomotive Behaviors

	N_O	% of category	N_F	% increase
Approach – Retreat (AR) Behaviors:				
Water Approach	105	55.0	38	23.8
Land Approach	38	19.9	21	15.4
Splash Entrance	23	12.0	19	13
Water Retreat	17	8.9	11	23.5
Land Retreat	8	4.2	5	12.5
Non Approach – Retreat (NAR) Behaviors:				
Body Bump	40	36.4	23	35
Slight Movement	20	18.2	11	20
Water Circle	19	17.3	13	21.1
Frontal Sit	16	14.5	13	68.8
Swim-by	7	6.4	8	28.6
Jump Over	4	3.6	4	25
Run-by	4	3.6	4	50

Locomotive behaviors are classified as AR or NAR behaviors. For each behavior, the table lists the total number of observations (N_O), the proportion of all observations within the category, AR or NAR (% of category), the total number of females observed to perform the behavior (N_F), and the proportion of observations in which each behavior was followed by an immediate increase in chuck number (% increase).

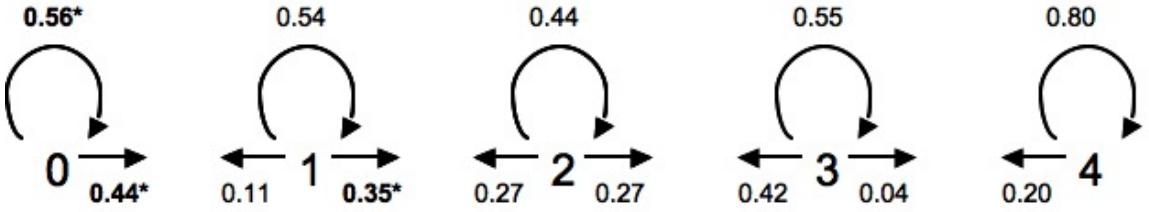
Male Response

Markov chain analysis of male calling bouts measured uncertainty as a zero, first, and second order process ($U_0 = 1.66$, $U_1 = 0.85$, $U_2 = 0.81$). The largest decrease in uncertainty occurred between the zero and first level measurements, which defined calling as a first order semi-Markovian process. This designation means that each call is best predicted by considering only the preceding call, thus analyzing call transitions as dyadic pairs is appropriate.

Presence versus absence of female movement has a significant influence on male response probabilities; the interaction of initial chuck number (0—4), transition type

(decrease, maintain, or increase), and female movement (present or absent) was significant in the model of male response strategy (GEE; N = 41 couples; Wald Chi-square = 279.9; df = 7; $p < 0.0001$; Figure 1.3). Pair-wise comparisons show that this difference is driven by a differential probability of decreasing and increasing rather than maintaining chuck number (decrease: $p < 0.0001$; maintain: $p < 0.871$; increase: $p < 0.0001$).

A) Transitions following female movement



B) Transitions not following female movement

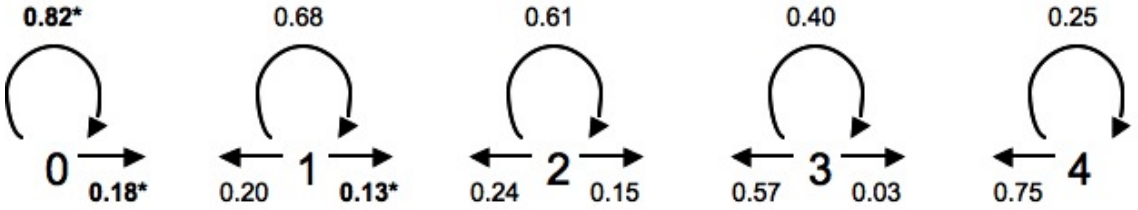


Figure 1.3: Female Movement and Call Transitions

Males change calling strategy when females move. Arrows show the mean proportion of transitions that decrease (←), maintain (↻), or increase (→) chuck number for initial chuck numbers of 0, 1, 2, and 3 (N = 41 males). The first set (A) represents those transitions made following female movement. The second (B) represents all other transitions in a call bout. Overall differences between A and B were significant (GEE; N = 41 couples; Wald Chi-square = 279.9; df = 7; $p < 0.0001$). Boldfaced individual probabilities significantly differ between A and B (*: $p < 0.0045$; GEE EM-Means pair-wise comparisons, using the Least Significant Difference setting. All 11 transitions were compared, so the significant p-value is derived from 0.05/11).

Males were most likely to increase chuck number when females produced NAR movements; the interaction of movement type (AR and NAR) and transition type (decrease, maintain, or increase) was significant in the model of male response strategy (N = 41 couples; Wald Chi-square = 9.841; df = 2; p = 0.007). Males are more likely to increase chuck number after NAR movements (p = 0.028), and are more likely to maintain chuck number after AR movements (p = 0.028; Figure 1.4). Results support the prediction that male response differs between elicitation (NAR) and mate acquisition (AR) behaviors.

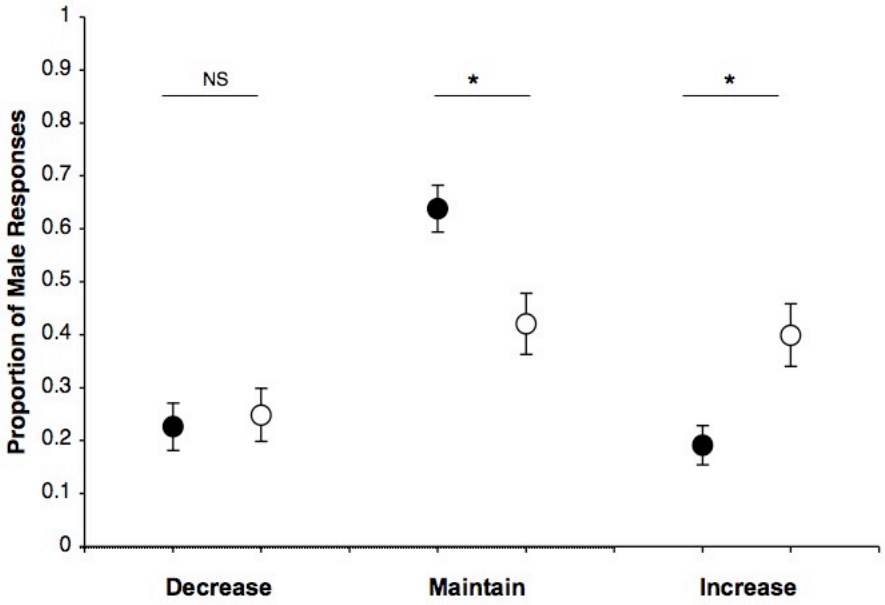


Figure 1.4: AR versus NAR Movements and Male Response

Circles show mean +/- SE proportion of responses that decrease, maintain, or increase chuck number when females move. Males (N = 41) increase chuck number more often following NAR movements (open circles), and maintain chuck number more often following AR movements (dark circles). *: $p < 0.03$

Number of Female Movements

We predicted that the number of males in a chorus would negatively correlate with the number of female movements. We found no correlation ($N = 30$; $r = -0.27$; one-tailed $p = 0.077$). Results do not support the prediction that elicitation behaviors are density-dependent.

We predicted that the largest chuck number produced prior to female movement would negatively correlate with the number of NAR but not AR female movements. There was a negative correlation for NAR movements ($N = 24$; $r = -0.36$; one-tailed $p = 0.04$) but not AR movements ($N = 24$; $r = -0.07$; one-tailed $p = 0.38$). The lower sample size results from some females moving immediately, which did not allow a comparison of pre-movement chuck number. Results support the prediction that prior chuck number influences number of NAR movements.

We predicted that the number of NAR but not AR movements would positively correlate with the increase in chucks between the first movement and amplexus. NAR but not AR movements were positively correlated with the increase in chuck number following the first movement ($N = 24$; NAR: $r = 0.54$; one-tailed $p = 0.003$; AR: $r = 0.31$; one-tailed $p = 0.07$). Results support the hypothesis that NAR movements elicit an increase in chuck number.

Complexity of Initial Call

Initial chuck number influenced the male's transition strategy; the interaction of initial chuck number and transition response was significant in the model of transition probabilities (GEE; $N = 41$ couples; Wald Chi-square = 136.8; $df = 7$; $p < 0.0001$). Also, initial chuck number and probability of increase are negatively correlated overall ($r = -0.23$; one-tailed $p = 0.0006$) and for the subset of transitions in response to female

movement ($r = -0.31$; one-tailed $p = 0.0019$). Results support the hypothesis that signal elaboration is increasingly costly to males.

DISCUSSION

This study demonstrates that túngara frog females possess a repertoire of elicitation behaviors that actively influence the dynamic display of mating signals towards a sexual selection optimal value. These elicitation behaviors do not move females directly towards or away from males, instead they appear to function to elicit more elaborate calls from males. Our results show that males are more likely to add chucks to their calls following female movement relative to the rest of a call bout, and they are more likely to add chucks following elicitation behaviors than following acquisition behaviors. Thus we conclude that females exhibit elicitation behavior to manipulate male display.

The elicitation (NAR) behaviors we describe are probably density-dependent according to male chorus size. Density-dependent mating behaviors are common (Kokko & Rankin 2006). Túngara frog elicitation behaviors specifically should be more common in low-density choruses. Since larger, high-density choruses produce more chucks (Bernal et al. 2007), the benefits of eliciting more chucks are reduced in large choruses. In this study, females tended to produce fewer movements when more males were in the chorus, but the relationship was not significant. The range of chorus sizes tested (1 - 4 males), however, samples only a small range of chorus sizes that occur naturally. The influence of chorus size on female movement would be better established with a wider range of chorus sizes.

The number of NAR but not AR movements performed by each female correlates with the greatest chuck number produced prior to movement, and with the increase in

chuck number between first movement and amplexus. Together, these results suggest that NAR movements have a special relationship with male production of chucks. This relationship occurs even though our experiment did not control for several potential sources of variation in female condition. For example, although all females were captured in amplexus, time since capture could influence how soon females would need to release their eggs. Also, variation in age and experience with mating might have influenced females, because data were collected across an entire breeding season during which females mate multiple times. These factors can influence variation in female mating behavior in some species (Jennions & Petrie 1997).

The probability of increasing chuck number negatively correlates with the initial number of chucks produced in a dyadic pair of calls. This pattern might occur because the benefit of more chucks diminishes as more chucks are added (Bernal et al. 2009), or because adding more chucks increases costs incurred from predation (R. A. Page, unpublished data). Even after females were introduced, males never produced the maximum known number of chucks appended to a call—seven. Whether all males can reach the same maximum number of chucks is unknown, but 5 chucks were the maximum in this study, so reaching 7 is quite rare. Energetic costs are probably not significant because adding chucks does not increase oxygen consumption (Bucher et al. 1982).

We do not know why females elicit signal elaboration from males. The immediate result is an increase in chuck number produced by the male, and a greater number of chucks could function to influence the female's or male's reproductive physiology, assist in mate assessment, or both. These possibilities could fit within three different evolutionary frameworks.

Chase-away selection is a hypothesis that explains the evolution of elaborate male traits, but it is also grounded in female sensory and reproductive physiology (Arnqvist & Rowe 2005; Holland & Rice 1988). This hypothesis predicts that when mating is costly, females should evolve stimulus response thresholds that males must surpass for mating to occur, and that this threshold should exceed the stimulus level produced by a certain proportion of males. The catch is that females with few potential mates and a limited window of time for reproduction risk reproductive failure. Female elicitation behaviors could be favored by selection to rescue females from reproductive failure in instances in which ovulation is imminent but potential mates are not exceeding her threshold of reproductive stimulation.

The Handicap Principle (Zahavi 1975; Zahavi & Zahavi 1997) asserts that females assess heritable variation in males by attending to costly displays; Zahavi & Zahavi (1997) point to the túngara frog and its complex calls as a classic example. It follows from this premise that females should evolve elicitation behaviors to test the degree to which males are willing to engage in costly behavior. A similar phenomenon can occur in the context of dominance signaling, for example in Harris sparrows badge size signals fighting ability, which can be tested by challengers in dominance interactions (Rohwer 1975). Zahavi & Zahavi (chapter 11, 1997) talk about "testing the social bond" in contexts besides mate assessment but the notion applies here as well. In the case of túngara frogs, adding chucks could force males into competition with neighboring males (Goutte et al. 2010), and females might judge the interactions that occur between males, as occurs in several bird species (Otter & Ratcliffe 2005).

Elicitation behaviors also fit within the framework of sexual stimulation influencing reproductive physiology (Adkins-Regan 2005; Lehrman 1965). Producing more chucks might acoustically stimulate hormone production in females, which could

influence her reproductive physiology or her motivational state in ways that increase the probability of successful mating. Behaviors that facilitate reproduction in conspecific individuals of the opposite sex occur across diverse taxa (Crews 1998). A variety of male courtship behaviors facilitate ovarian development and consequently reproductive state (Wingfield 2006), and acoustic signals specifically have been shown to influence female reproductive physiology in birds (Cheng 2008; Lehrman 1965). Social acoustic stimuli are known to trigger hormonal response in anurans (Wilczynski et al. 2005); the time course of these effects is usually thought to be on the orders of hours but few studies have examined effects at a smaller time scale.

A more circuitous advantage to elicitation behaviors might be in their effect on male sexual performance. Males might experience a process similar to behavioral efference, by which aggressive displays contribute to a feedback loop of increasing aggression in the signaler (Bond 1989). This scenario could apply to reproductive displays as well, if a male that produces more chucks self-stimulates the release of hormones that influence his own reproductive behavior. This could result in increased reproductive success for a male and for his mate. For example, males that produce more chucks might release more hormones that are active by the time the couple releases gametes, a few hours after forming amplexus. This could influence his ability to release more sperm, to hold on to a female more tightly, or to kick up a foam nest. Or, elicitation behaviors may actually elicit a hormonal response from males that incidentally causes increased chuck production. Male mice release some hormones in an immediate response to perceiving a receptive female (Bronson & Desjardins 1982). Adkins-Regan (2005) suggests that an immediate hormonal response to females such as this may contribute to changes in a male's attractiveness, mating stamina, or sperm mobilization.

Female elicitation behaviors are consistent with at least three disparate, although not mutually exclusive, hypotheses that attempt to explain the evolution of elaborate and costly courtship signals. Documenting the existence of elicitation behaviors does not allow us to discriminate among these and other hypotheses, but it does provide evidence of a previously unappreciated female behavior. These results indicate that despite a long history of recognition for the power of female mate choice in driving sexual selection (Trivers 1972) there is still an under-appreciation of the ways that females can actively influence male behavior (Clutton-Brock 2007; Gowaty 1997).

Behaviors that influence males to produce signals closer to a sexual selection optimum may be widespread in anurans and other taxa. Several anuran studies report courtship interactions including physical contact (Bourne et al. 2001; Kluge 1981; Ovaska & Rand 2001) or circling female movements (Owen & Tucker 2006). In a few cases anuran females even vocalize, which has been interpreted as advertising receptivity so males will approach the female (Tobias et al. 1998). Further studies may reveal that such female behaviors also act as elicitation behaviors. Active influence towards a sexual selection optimum is also likely in other taxa where males invest more energy into signaling (Patricelli & Krakauer 2010) when females are present. We expect that these female behaviors are common, because when females prefer costly signals, the sexual selection optimum signal value will always differ from the overall fitness optimum for a male. This perspective should influence models of escalating behaviors in dynamic interactions that predict low occurrence of escalation (Payne & Pagel 1996), because the potential costs and benefits of escalation in male-female courtship interactions differ from the costs and benefits in competitive or aggressive interactions.

In summary, female túngara frogs possess a repertoire of elicitation behaviors that effectively influence males to increase the number of chucks in their calls, thereby

pushing their signaling strategy towards the sexual selection optimum and increasing the costliness of mate advertisement.

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Chapter 2: Proximity-dependent response to variably complex mating signals in túngara frogs (*Physalaemus pustulosus*)

ABSTRACT

Behavioral plasticity allows individuals to maximize fitness in a wide range of contexts. Both production of, and preference for, mating signals are context-dependent according to internal factors such as hormonal state, and external factors such as predation risk. In many species, male to female proximity also defines an important context for mating communication. Males often possess short-distance courtship signals, and females often exhibit distance-related variation in signal response. Such variation in response may occur when a signal's relevance changes with male to female distance, but it may also result from perceptual constraints that are unrelated to fitness. Túngara frogs produce variably complex advertisement calls, and sexual selection theory predicts that females should prefer calls of greater complexity. Preference tests, however, have not demonstrated consistent trends for preference between calls of variable complexity. We tested whether proximity to males influences female response to variable signal complexity, and found that both preference and memory for signal complexity are proximity-dependent.

INTRODUCTION

Behavioral plasticity allows animals to modify decisions and achieve successful outcomes in variable environments. For example, animals produce and respond to mating-related communication signals with great variability according to context (Jennions & Petrie 1997; Lindstrom et al. 2009). Both external changes in the environment, such as predation risk (Bertram et al. 2004), and internal changes in an

individual, such as hormonal state (Lynch et al. 2006), influence receiver response to advertisement signals (Ryan et al. 2007). Proximity to potential mates is a contextual factor that dramatically influences mating-related communication. Males often respond to female proximity by changing the intensity of their display (Brumm & Slater 2006; How et al. 2008; Patricelli & Krakauer 2010), or by producing entirely different signals (Bourne et al. 2001; How et al. 2007; Titus 1998). Females generally prefer closer rather than more distant males (Booksmythe et al. 2008; MacLaren 2006), possibly because of potential costs of spending more time choosing a mate (Sullivan 1994). Proximity can also influence female response to mating signals by altering patterns of selectivity and discrimination (Beckers & Schul 2004; Doolan & Gerald 1985; Farris et al. 1997; Gerhardt & Schul 1999; Marquez et al. 2008).

We examine the influence of proximity in an attempt to understand female response to signal variation in túngara frogs (*Physalaemus pustulosus*). Túngara frog advertisement calls can have two parts: an initial low frequency sweep called a "whine," and a terminal high frequency burst called a "chuck" (Ryan 1985; Ryan & Rand 2003a). Whines may be voiced alone (simple calls), or followed by 1 to 7 chucks (complex calls). Females exhibit a strong preference for complex to simple calls [for 1-chuck: 86%, N = 3662 (Gridi-Papp et al. 2006)], yet previous studies have not found consistent patterns of preference for greater complexity, or more to fewer chucks (Bernal et al. 2009). Frog-eating bats (Ryan et al. 1982) and blood-sucking flies (Bernal et al. 2006) preferentially approach males that make complex calls, and bats preferentially approach males making more chucks over males making fewer chucks (R. A. Page, unpubl. data). Multiple chucks must benefit males in some way such that they continue to produce multiple chucks in spite of the costs associated with these calls. Thus the lack of consistent female

preference for greater complexity has called into question the function of multiple chucks.

Recently, we found that females in low-density choruses elicit greater complexity from males in close-range interactions (Akre & Ryan in prep), which suggests that multiple chucks may be most relevant to females at close range. In this study, we test whether proximity influences female preference for greater chuck number. Female working memory (Akre & Ryan 2010) and strength of commitment to approach a call (Baugh & Ryan 2010) increase for 3-chuck calls relative to 1-chuck calls, so chuck number is salient in some contexts. At close proximity to males, multiple chucks might be more meaningful, or they might stimulate female sensory systems to a greater degree. Both possibilities could contribute to a female response to calls with multiple chucks that is proximity-dependent.

The structure of anuran hearing organs supports the possibility that females require the greater stimulation that occurs at close proximity to males in order to distinguish number of chucks in a signal. Frogs have two inner ear organs with different frequency ranges to which they are most sensitive: the amphibian papilla (AP) is most sensitive to frequencies below 1200 Hz while the basilar papilla (BP) is most sensitive to frequencies above 1500 Hz. The AP has lower thresholds for response than the BP (Capranica 1976; Ryan et al. 1990). The addition of chucks to túngara frog calls adds energy in a higher frequency range to which the BP is most sensitive: about 90% of a chuck's energy is in frequencies > 1500 Hz (Ryan et al. 1990). Although females respond specifically to chucks at least as far as 3 m from a male (M. Ryan and S. Rand, unpubl. data), the ability to discriminate between chuck numbers might require greater BP stimulation. Frequency-dependent discrimination thresholds are reported in other anuran species (Gerhardt 1976; Gerhardt & Schul 1999).

We measured female preference for greater chuck number at three amplitudes representing three male to female distances. We also made predictions related to the mechanism that could explain a proximity-dependent response. First, if female discrimination is constrained by sound attenuation over distance, then increasing the energy in the chucks should increase the proportion of females that can discriminate between chuck numbers. Second, if females respond to call complexity at great distances with a non-discrimination task, such as memory, this would demonstrate an ability to perceive the signal at that distance.

METHODS

P. pustulosus were collected in Gamboa, Panama (9°07.0′ N, 79°41.9′ W) between September and November of 2007 and between June and September of 2008 and 2009. Male-female pairs in amplexus were brought to a Smithsonian Tropical Research Institute laboratory for testing, and then released after testing the same night at the site where they were originally collected. While at the laboratory, pairs were stored in dry dark containers to reduce stress and avoid moisture that could stimulate the female to drop her eggs. To avoid using the same female more than once in a given test, we followed standard toe-clipping procedures to mark individuals before release. Toe clip procedures followed the Guidelines for Use of Live Amphibians and Reptiles in Field and Laboratory Research, compiled by the Herpetological Animal Care and Use Committee (HACC) of the American Society of Ichthyologists and Herpetologists (http://www.asih.org/files/hacc-final.pdf).

We used female phonotaxis in a binary choice test as an assay for call preference and memory. We followed previously successful procedures with some modifications (Ryan 1985; Ryan & Rand 2003a). For each test, we placed a female under a funnel in

the center of a sound attenuation chamber measuring 2.7 x1.8 m, between two speakers (ADS L200C) placed at a distance of either 50 cm or 120 cm on either side of the female, depending on the experiment. Testing was monitored remotely with an IR illuminated camera (Fuhrman Diversified, Inc.). Each speaker broadcast 1 call every 2 sec, and calls from the two speakers were antiphonal. The funnel was lifted remotely, and once a female was released we scored her response as a choice for one of the speakers when she approached to at least 10 cm of that speaker within 10 min of being released from the funnel. If she did not reach a speaker within 10 min, if she failed to leave the center of the chamber within 5 min, or if she remained immobile for 2 min at any point in the test after leaving the circle, she was scored as "no choice." In addition to speaker choice, we recorded latency to leave the center of the chamber and latency to reach the chosen speaker. We predicted that latency would decrease as call amplitude increased.

We use call amplitude to represent proximity in our tests. Call amplitude changes predictably with distance from the source; this is the reason that many receivers use ranging, a process by which receivers attend to amplitude as an indication of distance (Morton 1986; Naguib & Wiley 2001). Although excess attenuation of calls occurs over distance, the closer a receiver's proximity to signalers, the more predictable the relationship between amplitude and distance. Several studies suggest that anuran response to variable call amplitude has consequences based on the relationship between amplitude and distance. Males respond more aggressively as neighboring males' call amplitude increases (Owen & Gordon 2005; Wells & Schwartz 1984). Call amplitude of neighboring males can also influence nearest neighbor distances in choruses (Brenowitz et al. 1984; Murphy & Floyd 2005). Females can adjust approach motor behaviors according to signal amplitude, such that each movement is shorter as amplitude increases (Beckers & Schul 2004). Some females are able to judge the amplitude of male calls

independent of distance (Castellano et al. 2004; Murphy 2008), but the above studies demonstrate that variability in call amplitude produced by males is not great enough to nullify the general correlation between amplitude and proximity. We have limited data on amplitude variation within túngara frog males, but in an analysis of 12 previously collected call bout recordings (Bernal et al. 2009), the amplitude variation within a call bout recorded at 1 m from a male is such that on average, the standard deviation of the whines' peak amplitude is +/- 10.7% of the mean.

Preference Tests

We tested whether female preference for greater complexity differs with call amplitudes that represent three male to female proximities. For these preference tests, one speaker broadcast a whine with 1 chuck and the other speaker broadcast a whine with 3 chucks. Calls were broadcast while the female was restrained under the funnel at the center of the arena, and after 3 min she was released to approach a speaker. The distance from the female to each speaker was 50 cm. A male's call is about 90 dB SPL (re. 20 μPascals for all sound pressure levels in this study) at 50 cm (Ryan 1985), thus for each amplitude we can estimate the corresponding natural female-male distance. Speakers broadcast calls such that peak amplitude level of the whine at the center of the chamber was 82 dB SPL, which corresponds with a female-to-male distance of about 1.3 m in nature, 90 dB SPL, which corresponds with the actual distance to the speakers, 50 cm, or 94 dB SPL, which corresponds with a female-to-male distance of about 32 cm in nature.

We first conducted the preference test using synthetic calls that were created to match the average call characteristics of the population (e.g. Ryan & Rand 1995; software developed by J Schwartz). For the synthetic calls, the chuck:whine amplitude ratio was 2.0, and the chuck duration was 45 ms.

We also conducted the experiment using variable natural calls to determine whether the amount of energy in the chuck series influenced female choice for 3-chuck calls. We used recordings from 20 different males that represent the natural variation of call properties in this population of túngara frogs (Ryan & Rand 2003b). These natural calls all consisted of whines with 1 chuck, and are the same calls used in Bernal et al. (2009). Natural calls vary along many call parameters, whereas synthetic calls were simplified constructions of the population average along 7 call parameters. We tested whether the total energy of the chucks for each natural call correlated with the number of choices for more chucks (range: 0 – 3 choices). For the natural calls, the chuck:whine amplitude ratio ranged from 0.18 to 2.21 (mean +/- SD = 1.07 +/- 0.49). The chuck duration for natural calls ranged from 32.9 to 115.5 ms (mean +/- SD = 53.7 +/- 19.5). This was a repeated-measures study and treatment order was randomized among females.

Memory Tests

We tested whether female memory for 3-chuck calls differs with call amplitude. For these tests, both speakers broadcast whines for 1 min, then for 30 s one speaker broadcast whines with 3 chucks while the other continued broadcasting a whine alone. Following this period, the speakers ceased to broadcast sound for a silent duration of 30 s, after which both speakers again broadcast whines alone, and we released the female to approach a speaker. A previous study showed that when females are tested at 90 dB SPL and speakers are 50 cm away from the center of the chamber, females significantly choose the speaker that originally played chucks (Akre & Ryan 2010). We repeated this memory test at 82 dB SPL with the speakers 120 cm away from the center of the chamber to match the natural distance at which males would produce this call. We also repeated this memory test at 82 dB SPL with the speakers 50 cm away from the center of the

chamber to control for the distance difference in the above condition. We compared these results to the previously published data. For these experiments, each female was used for only one choice.

Statistics

To analyze the influence of amplitude on preference for greater complexity, we used the Generalized Estimating Equations (GEE) procedure in SPSS 16® to create a binary logistic model of speaker choice predicted by call amplitude with test order. We created separate models for synthetic and natural calls. For the tests using synthetic calls, females completed a series of tests in random order, and our statistical analysis controlled for the fact that most females completed multiple tests, but few completed all conditions, by using the repeated subject function. For the tests using natural calls, each of 20 females completed all three amplitude conditions in a repeated measures design. Each female was presented with a different natural call for each amplitude condition, and each of 20 natural calls was used once at each of the three amplitudes. Pair-wise comparisons were conducted with the EMMeans function using a sequential Bonferonni adjustment for multiple comparisons.

We also used the GEE procedure to create linear models with latency as a dependent variable. We created separate models for tests of synthetic and natural calls, modeling latency as both the total time before females made a choice and the time before females left the center of the chamber. In both cases, the independent predictors were call amplitude and test order.

To analyze the influence of amplitude and distance on outcome in the memory tests, we used a separate group of females to test each condition. We tested for memory

in each condition using an exact binomial test, and compared results between conditions with a fisher's exact test.

RESULTS

Preference Tests

In the test of synthetic calls, a logistic regression using the generalized estimating equation (GEE) with call choice as the dependent variable and order and call amplitude as the predictors showed that call amplitude was a significant factor in the model (N = 177 choices, 107 females; Wald Chi-Square = 6.403; df = 2; p = 0.041; Figure 2.1a). Order of signal presentation was not a significant factor (p = 0.102). Pair-wise comparisons with EM Means (using the sequential Bonferroni setting) showed choice to be significantly different for call amplitudes of 82 and 94 dB SPL (p = 0.029). Latency decreased significantly as call amplitude increased, both when measured as the total time before females made a choice (Wald Chi-Square = 19.017; df = 2; $p < 0.001$), and when measured as the time before females left the center of the chamber (Wald Chi-Square = 17.911; df = 2; $p < 0.001$).

In the test of natural calls using the GEE analysis, call amplitude was a significant predictor of speaker choice (N = 60 choices, 20 females; Wald Chi-Square = 13.301; df = 2; $p < 0.001$; Figure 2.1b). Order of signal presentation was not a significant factor (p = 0.675). Pair-wise comparisons showed choice to be significantly different for call amplitude of 82 and 94 dB SPL ($p < 0.0001$) as well as 90 and 94 dB SPL (p = 0.01). Full-test latency was not predicted by call amplitude (Wald Chi-Square = 0.148; df = 2; $p < 0.929$), nor was latency to leave the center of the chamber (Wald Chi-Square = 5.187; df = 2; $p < 0.075$).

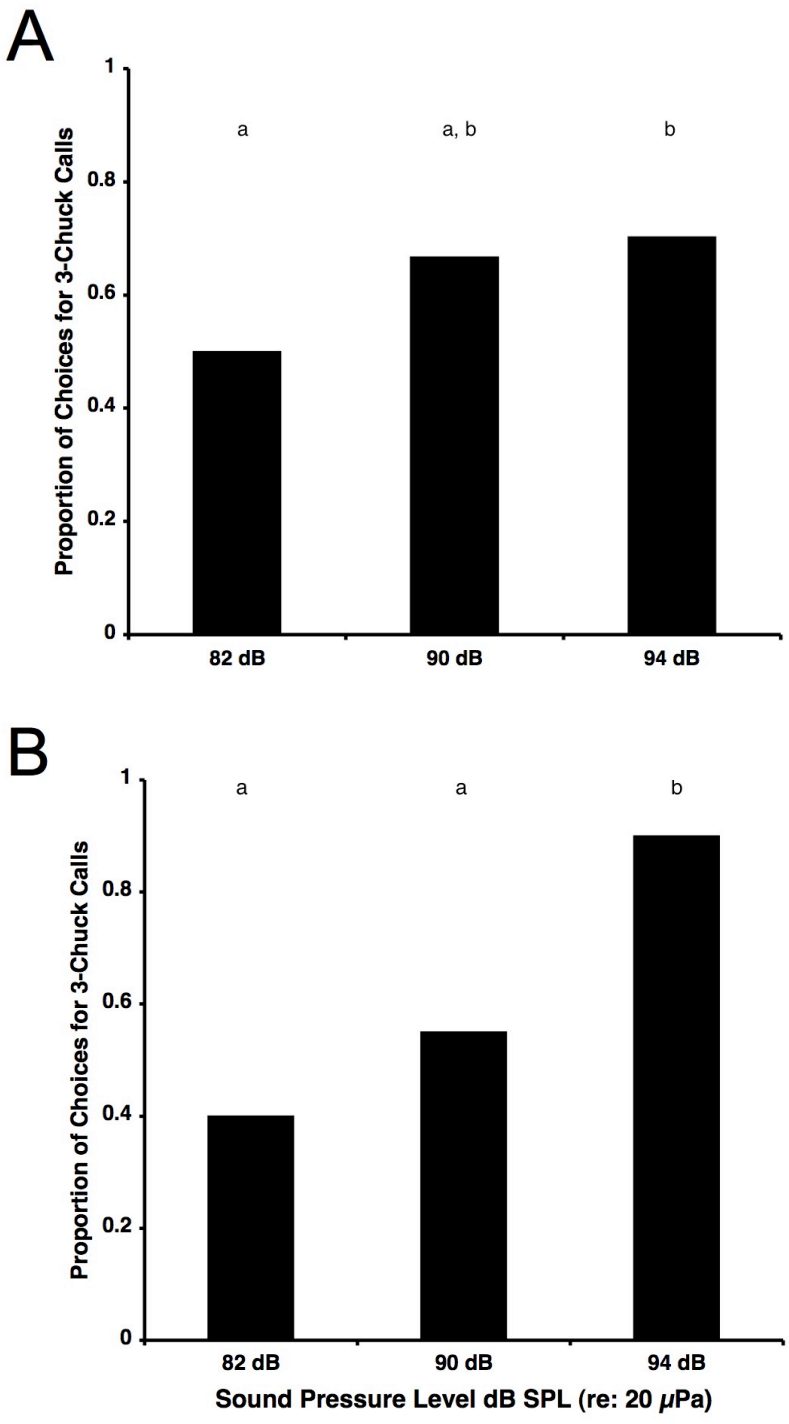

Figure 2.1: Amplitude and Female Preference For Greater Complexity

Female preference for 3-chuck calls over 1-chuck calls increases with call amplitude in tests of A) synthetic calls and B) natural calls. Bars show proportion of females that chose the 3-chuck call. Letters mark pair-wise significance.

The strength of preference for greater complexity did not differ between synthetic and natural calls (2-tailed Fisher's Exact; 82 dB: $p = 0.606$; 90 dB: $p = 0.424$; 94 dB: $p = 0.130$).

The total energy in the natural chuck did not have a significant correlation with number of choices for greater chuck number (N = 60 choices; $R^2 = 0.11$, $p = 0.403$).

Memory Tests

Females did not exhibit memory for the speaker that originally played chucks in the 82 dB condition at the natural distance of 120 cm (Exact binomial two-tailed: N = 41 females; $p = 0.349$) or at 50 cm (N = 43 females; $p = 1.0$; Figure 2.2), and the responses at these two distances were not significantly different from one another ($p = 0.519$). The response to calls at 90 dB (previously reported in Akre & Ryan 2010) was significantly different than both 82 dB conditions (Fisher's Exact two-tailed: at 120 cm, $p = 0.003$; at 50 cm, $p = 0.023$).

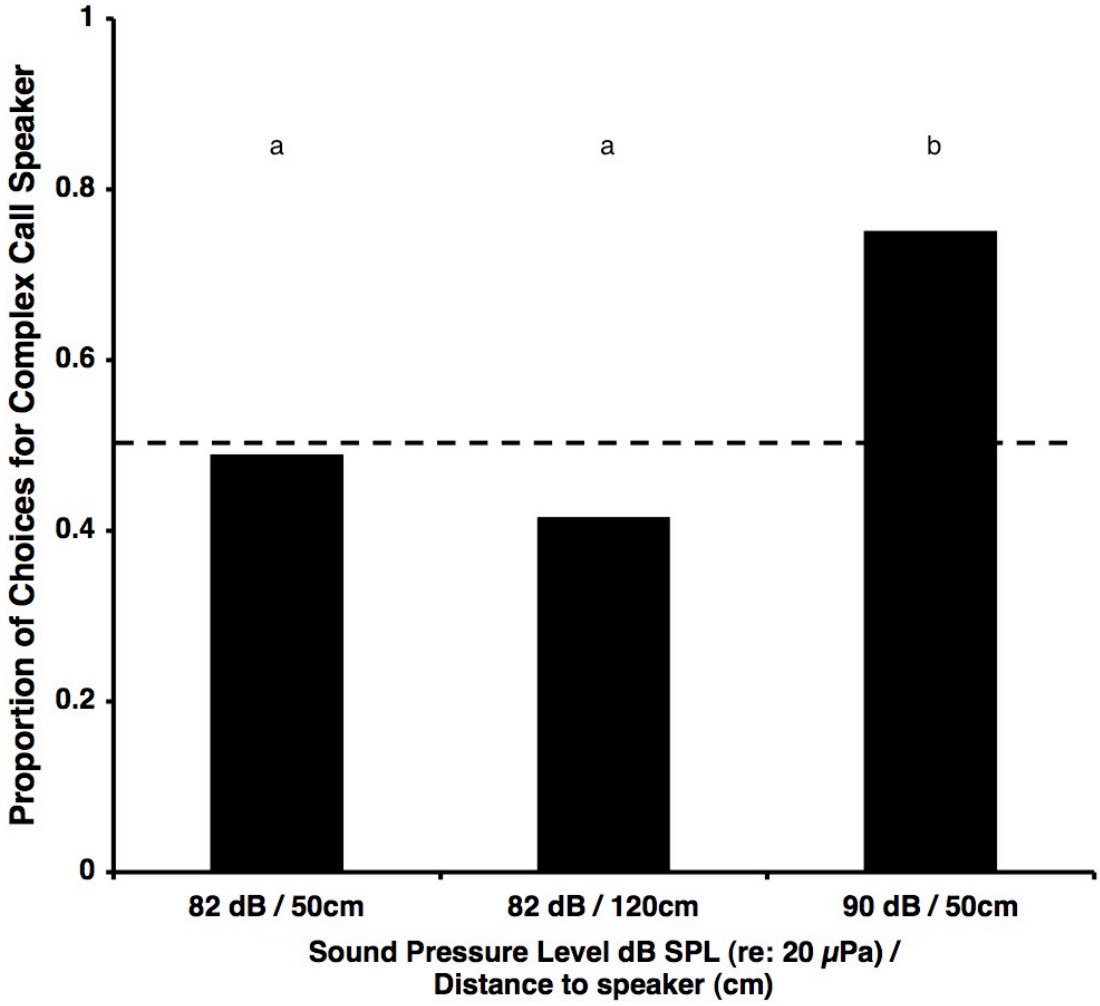

Figure 2.2: Amplitude and Female Memory for 3-chuck Calls

Memory for 3-chuck calls occurs only for high amplitude calls. Bars show proportion of choices for the speaker that originally played 3-chuck calls. Dashed line shows null hypothesis of 50%. Letters show pair-wise comparison significance.

DISCUSSION

Call amplitude, which co-varies with proximity, influences both female preference and memory for call complexity. In preference tests between calls with 1 and 3 chucks, preference strength for 3-chuck calls increased with call amplitude for both

synthetic and natural calls. Thus chuck number influences mate choice at close proximity, and female preference for greater complexity could explain why males produce multiple-chuck calls in spite of the increased predation risk associated with those signals.

We do not know the mechanism for amplitude-dependent difference in female response to chuck number. The pattern we find here, that is, more discrimination and memory at higher amplitudes, could result from the lower thresholds of the BP relative to the AP (Ryan et al. 1990). Alternatively, the critical difference could occur in the brain during neural processing of stimuli. In this case, females could perceive differences in chuck number at lower amplitudes and longer distances from the sender but have no reason to respond differently. The amount of energy in the chuck series did not correlate with number of choices for 3-chucks, but several points lead us to think that the more parsimonious interpretation of our data is that females do not discriminate chuck number at lower amplitudes because they cannot. 1) Amplitude influences both preference and memory, which are two different cognitive processes. 2) Our data match previous findings that frequency influences anuran amplitude threshold for discriminating temporal call features (Gerhardt 1976; Gerhardt & Schul 1999). 3) Once females do discriminate between chuck numbers at close range (higher amplitudes), the response follows Weber's law (Akre et al. in prep, Chapter 3), suggesting that discrimination is physiologically constrained even at high amplitudes.

Call amplitude influenced not only discrimination and memory, but also both latency to leave the starting position and latency to make a choice. This was true even though the distance from the females' starting position to the speaker was the same for each condition. One interpretation of this result is that females are generally more responsive to males that produce high amplitude calls. Responsiveness increases with

call amplitude in other anurans (Beckers & Schul 2004). Females prefer to approach a speaker that is louder at the source when it is paired with a closer speaker that is quieter at the source, but equally loud at the female's starting point (M. J. Ryan & A. Lea, unpubl. data). Amplitude-dependent latency, however, was only apparent in the tests that used synthetic calls. In the tests of natural calls, variation in multiple call properties extinguished the influence of amplitude on latency, which suggests that call amplitude at the source is weighed along with multiple call variables in mate choice decisions.

Other studies of anuran response to mating calls have found similar patterns of increased discrimination between call features at high amplitude (Gerhardt 1976; Gerhardt & Schul 1999). The influence of amplitude on discrimination, however, depends on what type of call feature is being discriminated. For example, amplitude has an opposite influence on discrimination acuity for differences in amplitude itself. At high sound levels, the ability to discriminate between two different amplitudes decreases in insects and anurans, although birds and mammals show the opposite trend (Wyttenbach & Farris 2004). Discriminating between chuck numbers may depend on female processing of duration, total energy, or BP-specific call energy.

This proximity-dependent response of receivers might occur commonly in a variety of communication systems. To better describe these proximity-related communication dynamics, a signal's active space can be described as a set of stratified layers. Active space is the area over which a signal influences a receiver's response (Marten & Marler 1977; Marten et al. 1977; Morton 1975; Wiley & Richards 1978). If a signal elicits a repertoire of different responses according to proximity, then the distance over which each response occurs can be envisioned as a series of stratified layers spreading from the sender's location (Figure 2.3). For example, in túngara frogs the female behavioral threshold for approaching multiple-chuck calls is around 61 dB SPL,

which corresponds to a distance of about 12 m (R. Taylor et al. in prep). The threshold for discriminating multiple-chuck calls from whines is around 72 dB SPL, which corresponds to a distance of about 3 m (M. Ryan & S. Rand, unpubl. data). The threshold for remembering 3-chuck calls and for discriminating 3-chuck and 1-chuck calls is around 90 dB, which corresponds to 50 cm. So the first stratum of active space for 3-chuck calls is 50 cm in diameter, the next is a diameter of 3 m, and the third is about 12 m in diameter. A measurement of active space for each receiver response to a signal could improve our understanding of how the environment influences signal evolution.

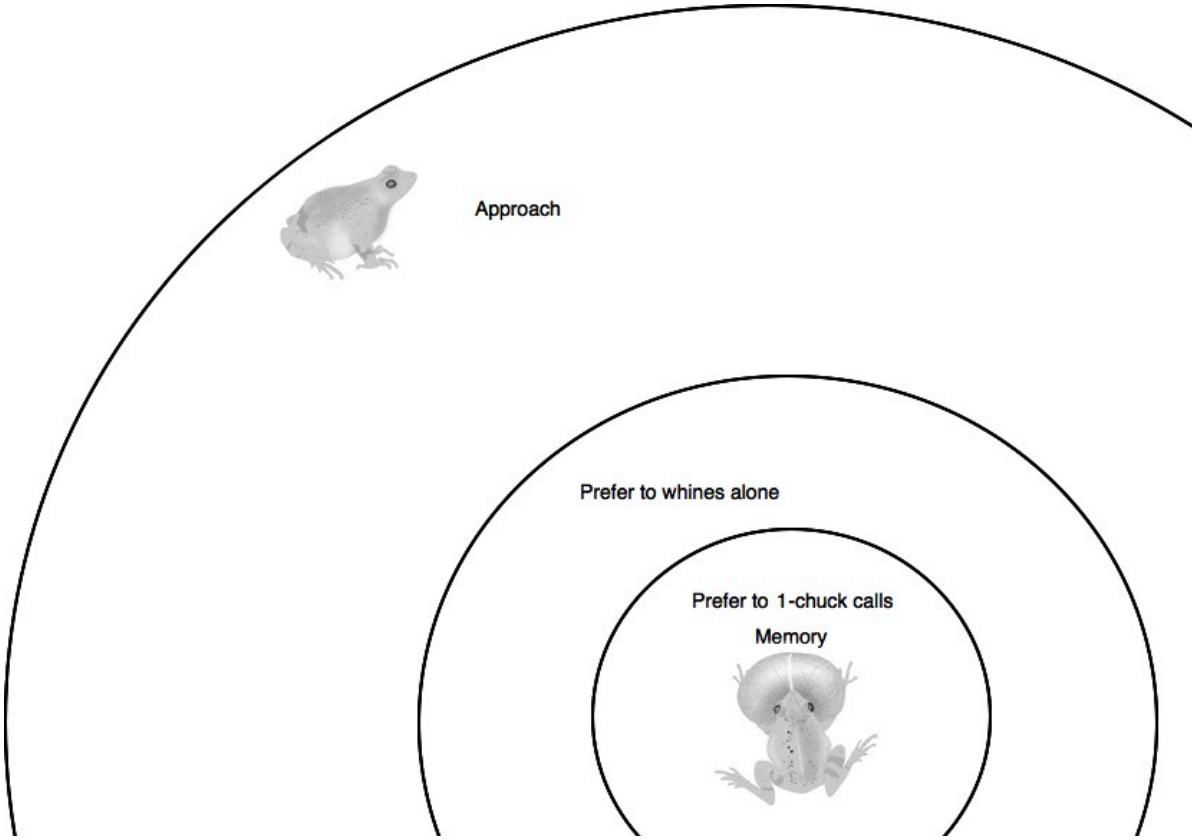

Figure 2.3: Stratified Active Space

This stratified active space depicts the area over which a male's 3-chuck call stimulates each response in the female's response repertoire.

In summary, we show that signal amplitude, and therefore male to female proximity, influences female túngara frog response to mating signals. Female preference for more to fewer chucks at close proximity explains why males produce calls with multiple chucks in spite of the increased predation risk associated with those calls.

Acknowledgements

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Chapter 3: Preference for a sexually selected trait follows Weber's law

ABSTRACT

Elaborate male traits evolve through sexual selection, yet the extent to which traits become exaggerated is not unchecked. Natural selection, pleiotropy, and species recognition can limit signal elaboration. Reduced female response to extremes might also curb trait elaboration, yet when species recognition is not at stake, the reason for reduced female response is unclear. Weber's law might explain how the receiver's perceptual biology causes a diminished female response to increasing elaboration. This law states that the ratio between stimulus magnitude and the threshold difference at which two stimuli can be discriminated is fixed. Thus discrimination between stimuli differing by a fixed amount becomes more difficult with increasing stimulus magnitude. If females are constrained by Weber's law, discrimination between stimuli could decrease as elaboration increased, and this could reduce the benefits of increasing elaboration. We proposed that such phenomena might explain female túngara frog response to male trait variation and found that female discrimination matches the predictions of Weber's law. Thus the cognitive machinery of female choice can contribute not only to the evolution of elaborate male signals, but also impose limitations on this evolutionary process.

INTRODUCTION

Sexual selection is responsible for the evolution of exaggerated male traits (Andersson 1994; Darwin 1871; Ryan & Keddy-Hector 1992), yet such exaggeration does not proceed unchecked. Natural selection can impose costs on elaboration, or exploited females might evolve resistance to more elaborate traits (Arnqvist & Rowe 2005; Lande 1981). Either situation could favor males with reduced trait elaboration.

Alternatively, increased elaboration could be constrained by species recognition or by sexually antagonistic pleiotropy (Price & Burley 1994; Pryke & Andersson 2008). Another, less appreciated factor is diminished strength of female response as a function of increased trait elaboration (Bernal et al. 2009; Cohen 1984; Jennions & Petrie 1997). Why this diminished response might occur is unclear. One possible explanation is that females are constrained by their perceptual biology in the ability to distinguish stimuli (Cohen 1984; Ryan 1990).

Research in human psychophysics has a rich history of relating true stimulus value to perceived stimulus value. An important generalization of these studies is that the two do not scale to one another in a simple linear fashion. The essence of this finding is encapsulated in Weber's law (Yost 1994). Weber's law states that $(\Delta I / I) = k$, where ΔI is the threshold amount of change in stimulus magnitude necessary for a noticeable difference from a stimulus of magnitude I, and where k is a constant. Therefore, discrimination between two stimuli differing by a fixed amount becomes more difficult with increasing magnitude of the stimuli—a greater difference is required to discriminate between high magnitude stimuli.

Cohen (1984) suggested that Weber's law could influence the evolution of male traits due to its constraint on female preferences. We know that receiver response to amplitude of acoustic communication signals can follow Weber's law (Wyttenbach & Farris 2004). There are no data, however, showing that female preferences for male traits known to be under sexual selection follow Weber's law, although a few studies have shown this is not the case (Bee 2008; Gerhardt et al. 2000). If Weber's law does constrain female choice, this could explain why females might prefer elaborate signals but show diminishing response to increasing elaboration (Cohen 1984).

The system

We investigated Weber's law and female choice in túngara frogs (*Physalaemus pustulosus*). Túngara frog advertisement calls can have two parts: an initial low frequency sweep called a "whine," and a terminal high frequency burst called a "chuck" (Ryan 1985; Ryan & Rand 2003a). Adding a chuck increases total call energy by 8.9%. Whines may be voiced alone (simple calls), or followed by 1 to 7 chucks (complex calls), but calls with 2 or fewer chucks make up 99% of the calls produced generally (Bernal et al. 2007), and 87 % of the calls produced when females are present (Akre & Ryan unpubl. data). We refer to complexity as the number of components in the call; thus calls with more chucks are more complex. Males call in choruses, and a male usually produces both simple and complex calls of variable chuck number within a calling bout (Bernal et al. 2007). Males' call transitions occur in steps of +/- 1 chuck (Bernal et al. 2009), and males change the number of chucks produced in response to both neighboring males' calling strategies (Goutte et al. 2010) and female movements (Akre & Ryan in prep, Chapter 1). Females exhibit a strong preference for complex to simple calls [for 1-chuck complex calls: 86% of females choose the complex call, N = 3662 (Gridi-Papp et al. 2006)]. Females remember 3-chuck calls over short silent periods (Akre & Ryan 2010), exhibit a stronger commitment towards 3-chuck calls in a dynamic call environment (Baugh & Ryan 2010), and prefer 3-chuck calls over 1-chuck calls at close proximity to males (Akre & Ryan in prep, Chapter 2).

Since females prefer 3-chuck calls to 1-chuck calls at close range, we predicted that females in close proximity to males generally prefer more chucks to fewer chucks. Males rarely produce calls with more than 2 chucks, however, so we also predicted that females show diminishing ability to discriminate between chuck numbers as complexity (the number of chucks) increases. Though it is not possible for us to determine whether

female túngara frogs are unable to discriminate between chuck numbers or simply don't respond to signals that they perceive as different (Buckingham et al. 2007; Wyttenbach & Farris 2004), a response pattern following Weber's law would be strong evidence that female's are constrained by their perceptual biology in choosing the greater chuck number (Shettleworth 1999).

METHODS

P. pustulosus were collected in Gamboa, Panama between June and November of 2007, 2008, and 2009. Male-female pairs in amplexus were brought to a Smithsonian Tropical Research Institute (STRI) laboratory for testing, and then released the same night at the site where they were originally collected. While at the laboratory, pairs were stored in dry dark containers to reduce stress and avoid moisture that could stimulate the female to drop her eggs. To avoid using the same female more than once in a given test, we followed standard toe-clipping procedures to mark individuals before release. Toe clip procedures followed the Guidelines for Use of Live Amphibians and Reptiles in Field and Laboratory Research, compiled by the Herpetological Animal Care and Use Committee (HACC) of the American Society of Ichthyologists and Herpetologists (http://www.asih.org/files/hacc-final.pdf).

We used female phonotaxis in a binary choice test as an assay for call preference. We followed previously successful procedures with some modifications (Ryan 1985; Ryan & Rand 2003a; Akre & Ryan in prep). For each test, we placed a female under a funnel in the center of a sound attenuation chamber measuring 2.7 x 1.8 m, between two speakers (ADS L200C) placed at a distance of 50 cm on either side of the female. Each speaker broadcast 1 call/2 s, and calls from the two speakers were antiphonal. Peak amplitude of the whine was 90 dB SPL (re: 20uPascals) at the female's release point.

Testing was monitored remotely with an IR illuminated camera (Fuhrman Diversified, Inc.).

We tested females with pairs of calls that varied in chuck number as follows: 1:2, 1:3, 1:4, 1:5, 2:3, 2:4, 3:4. Throughout this study the less preferred stimulus (which is always the stimulus with fewer chucks) is Stimulus 1 (S1) and the more preferred stimulus (which is always the stimulus with more chucks) is Stimulus 2 (S2). We presented 1 min of whines alone followed by 30 s of the paired complex calls, after which we lifted the funnel so the female could approach a speaker. We used synthetic calls that were created to match the average call characteristics of the population (e.g. Ryan & Rand 1995; software developed by J Schwartz). The funnel was lifted remotely, and once a female was released we scored her response as a choice for one of the speakers when she approached to at least 10 cm of that speaker within 10 min of being released from the funnel. If she did not reach a speaker within 10 min, if she failed to leave the center of the chamber within 5 min, or if she remained immobile for 2 min at any point in the test after leaving the circle, she was scored as "no choice."

Weber's law

If females prefer more chucks but are constrained by the ability to discriminate chuck number, they should follow Weber's law. In this case, the proportion of females choosing S2 should decrease along a logarithmic curve as the ratio of chuck numbers in the stimuli (S1/S2) increases. That is, if the threshold change required to notice a difference from S2 is a constant proportion as predicted by the equation $(\Delta I / I) = k$, then as S1's proportional difference from S2 increases, the discrimination should get easier. Thus in tests of the proportional relationship S1/S2 ranging from zero to one, fewer females should be able to choose S2 as S1/S2 reaches closer to one.

The proportion of choices for the more preferred signal (S2) is bound by the values 0.5 and 1—it will never be less than 0.5 because then it would not be more preferred. Because our dependent variable is a measure of proportions, the confidence intervals (CIs) are binomial, and unequal above and below a given proportion. Due to the nature of these data, the appropriate model for testing Weber's law, as derived by Green et al. (1989), is:

$$proportion = \frac{1}{n} + \left[1 - \frac{1}{1 + e^{a*(m-x)}}\right] * \frac{n-1}{n}$$

where n is the number of possible choices (for binary choice tests $n = 2$); m is the mean of the distribution (where the proportion is set to equal 0.75, midway between 0.5 and 1; in this case $m = 0.208$); and a is the slope of the psychometric function (in this case $a = 4.298$). We used the equation to model predicted proportion outcomes for a series of independent variables increasing by 0.01 and ranging from 0.01 to 1, then compared our actual data with this model.

We included previously published data on the following discrimination tests: 0:1 and 0:3 chucks (Akre & Ryan 2010) to expand the range of S1/S2 ratios. These tests were conducted under the same conditions as the current data, using different females.

We also calculated stimulus magnitude as the total energy of entire vocalizations, including energy from both the whine and chuck series. To plot S1/S2 in terms of total call energy, we measured the relative sound energy in S1 and S2. For each stimulus, we summed the positive amplitudes of all time points within the amplitude envelope using Signal 5.0®. We then calculated the S1/S2 ratio for each test (Table 3.1).

Although Weber's law is based on stimulus perception of an individual, data on population level responses have been used repeatedly to test these predictions (Farris et al. 1998; Wyttenbach & Farris 2004). We know that population response proportions accurately predict individual responses for simple versus complex calls (Kime et al. 1998).

RESULTS

The more attractive stimulus always had more chucks, but the preference was not always significant (One-tailed Exact Binomial tests; 1:2, $N = 40$, $p < 0.135$; 1:3, $N = 41$, $p < 0.003$; 1:4, $N = 40$, $p < 0.004$; 1:5, $N = 40$, $p < 0.041$; 2:3, $N = 40$, $p < 0.319$; 2:4, $N = 40$, $p < 0.077$; 3:4, $N = 40$, $p < 0.318$; Figure 3.1). Significance was defined as $p < 0.007$, using a Bonferroni correction to account for conducting 7 tests at a $p = 0.05$ value. Table 1 lists choice results for each stimulus pair.

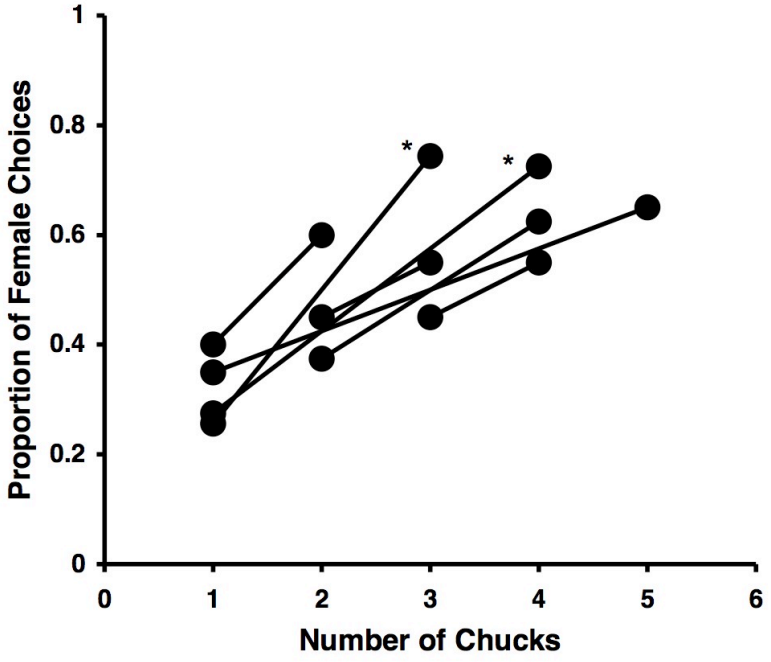

Figure 3.1: Female Preference Between Chuck Numbers

Females always prefer the greater number of chucks, but the preference is not always significant (* = $p < 0.007$). Lines connect proportion of female choices for each stimulus in a binary test.

Table 3.1: Female Response to Stimulus Pairs of Variable Chuck Numbers

Stimulus 1 (S1, chucks)	Stimulus 2 (S2, chucks)	Choices for S1	Choices for S2	S1/S2 (chucks)	Proportion Attracted to S2	S1/S2 (total energy)
0	1	4	21	0	0.84	0.92
0	3	2	23	0	0.92	0.79
1	5	14	26	0.20	0.65	0.75
1	4	11	29	0.25	0.73	0.80
1	3	10	31	0.33	0.76	0.86
1	2	16	24	0.50	0.60	0.93
2	4	15	25	0.50	0.63	0.87
2	3	18	22	0.67	0.55	0.93
3	4	18	22	0.75	0.55	0.93

Weber's Law

The results from the preference experiments significantly fit the logarithmic curve expected if females were constrained by Weber's law (N = 331 choices, 151 females, 9 stimulus pairs; $t = 6.156$, $p < 0.0005$, $R^2 = 0.844$; Figure 3.2). There was a strong preference for more chucks when S1/S2 was small (indicating a large difference between S1 and S2), and a weaker preference as S1/S2 increased (indicating a small difference between S1 and S2).

When we used relative call energy instead of chuck number as the measure of signal magnitude (independent variable), the results were not consistent with Weber's law (Figure 3.3). The ratio of call energy in two competing stimuli (S1/S2) ranged from 0.75—0.93, and the proportions of S2 choices showed no pattern through that limited range. Although addition of a single chuck to a whine results in a large increase in proportion of choices for that call (from 0.50 to 0.84) this is accomplished with the addition of only a small amount of call energy— 8.9%.

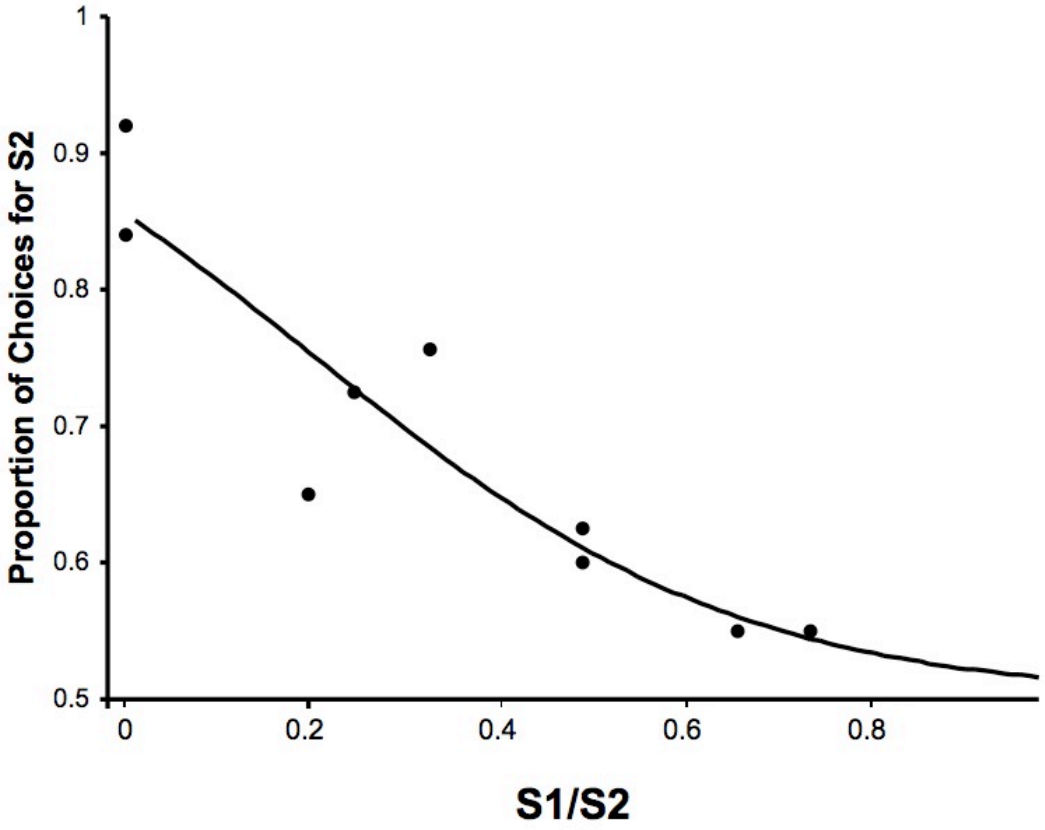

Figure 3.2: Fitting the Model of Weber's Law

Proportion of females that choose S2 significantly fits the model predicted by Weber's law. Line shows response proportions expected by Weber's law. Points show female response proportion choosing S2, which was the greater number of chucks in every case.

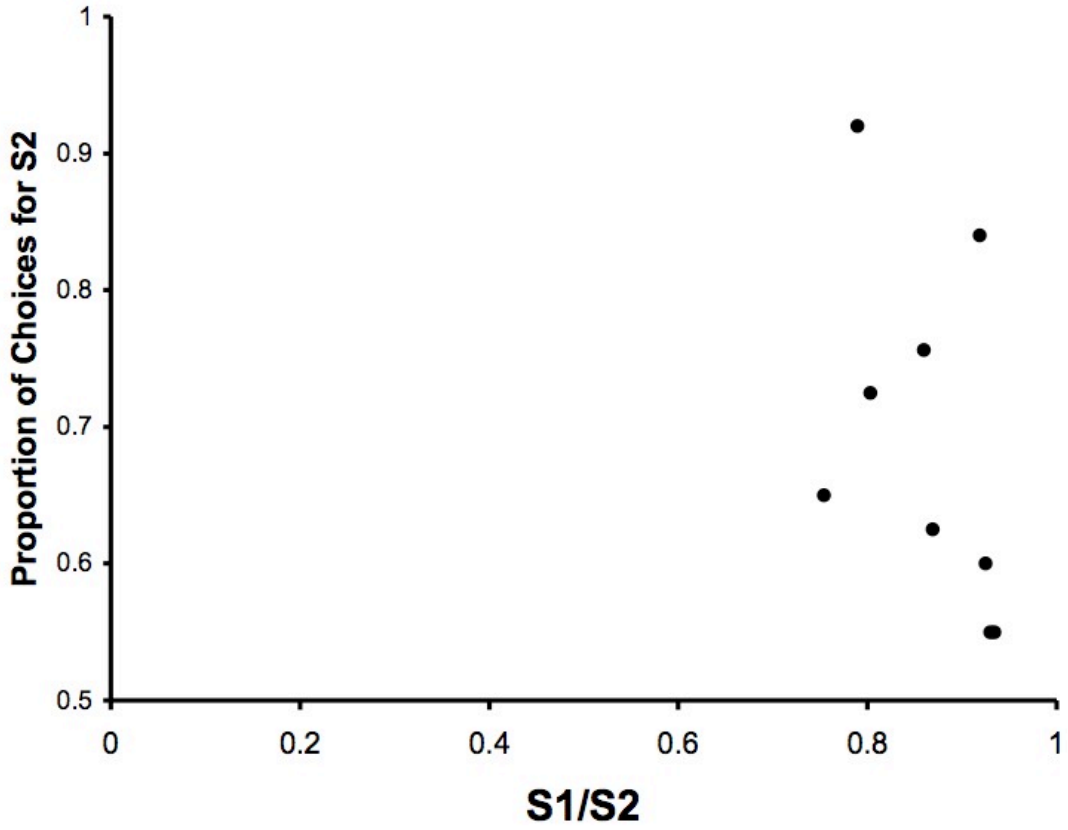

Figure 3.3: Stimuli as Total Call Energy

When stimulus magnitude is measured as total call energy, including both whine and chucks, response proportions do not adhere to Weber's law.

DISCUSSION

The evolution of complex calls in túngara frogs is a classic example of how sexual selection by female choice promotes trait elaboration which is, in turn, limited by costs of predation and parasitism imposed by eavesdroppers (Ryan in press). Here, we show that the female's cognitive biology might also constrain the unchecked elaboration of complex calls.

The relationship between strength of preference and ratio of chucks supports the assertion that females discriminate between multi-chuck signals according to Weber's law, but does not prove it. It is possible that females perceive all differences in chuck number but do not act on all differences because there is no reason to do so; that is, it is possible that we have measured just meaningful differences rather than just noticeable differences (Nelson et al. 1990). The more likely explanation is that females actively discriminate and favor more chucks when they are able to perceive differences. Female preference functions can take many forms (Kirkpatrick et al. 2006; Ritchie et al. 2001; Shettleworth 1999; Wagner 1998), so matching the predictions of Weber's law with an R^2 of 84% for reasons other than physiological constraint would be surprising, but still possible. In addition, more complex calls have more chucks and more call energy. Female preferences for chuck number follow Weber's law, but preferences for call energy do not. These results suggest that the preference for more complex calls is more than merely a preference for more call energy, a result that has also been demonstrated experimentally by Ryan and Rand (1990).

Trait elaboration by sexual selection is typically thought to be checked by costs that result from predation risk, energy expenditure, or error in species recognition (Fisher 1930; Kemp et al. 2008; Lande 1981; Pryke & Andersson 2008). This study demonstrates that exaggeration might also be limited as a result of reduced benefits imposed by physiological constraints on trait discrimination in females, as suggested by Cohen (1984). Interestingly, if predators that use conspicuous signals to locate prey are likewise constrained by Weber's law, this could diminish the costs imposed by predators on trait elaboration.

Studies of animal discrimination have used Weber's law to make and test predictions about signal detection (Endler et al. 2005), foraging (Bateson & Kacelnik

1995; Kacelnik & Bateson 1996), shoaling (Buckingham et al. 2007), and mating preferences (Bee 2008; Cohen 1984; Gerhardt et al. 2000; Shettleworth 1999; Wyttenbach & Farris 2004). Our study is the first that we are aware of to demonstrate that female preference for elaboration follows Weber's law and thus might limit the evolution of increasing trait elaboration. As others have predicted (Cohen 1984; Ryan 1990; Jennions & Petrie 1997; Shettleworth 1999), Weber's law probably influences signal evolution in many taxa as it draws from consistent properties of perceptual systems. Thus the cognitive machinery of females can contribute not only to the evolution of elaborate male signals, but also to limiting that same elaboration.

Acknowledgements

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Chapter 4: Complexity increases working memory for mating signals[*]

SUMMARY

Females often prefer to mate with males who produce complex signals (Andersson & Simmons 2006; Darwin 1871; Ryan & Keddy-Hector 1992). It is not clear why they do so. Females might prefer complexity if it predicts mate quality (Searcy & Nowicki 2005; Spencer et al. 2003; Hebets & Papaj 2005), or signals might evolve complexity to exploit females' sensory or cognitive biases (Hebets & Papaj 2005; Hartshorne 1973; Searcy 1992; Stripling et al. 1997). We tested whether complexity increases active time, the period over which a signal influences a receiver's response to that signal. Mating signals are often ephemeral, yet their active time has largely been ignored. Here we demonstrate that signal complexity influences active time in túngara frogs. Male advertisement calls consist of frequency sweeps (whines) followed by 0–7 high-frequency bursts (chucks). Females preferentially approach complex (whines with chucks) over simple (whines alone) calls but do not consistently prefer greater complexity (Bernal et al. 2009), so the function of multiple chucks has been uncertain. We found that females remember which speaker previously broadcast complex calls when choosing between simple calls broadcast after a delay. This effect occurred for calls with multiple chucks, but not with single chucks. Neither motivation nor orientation behavior differed with chuck number, suggesting that results are due to differential memory. Thus, female memory could favor the evolution of increasing signal complexity through sexual selection.

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RESULTS

Natural Inter-chorus Intervals

We measured the duration of túngara frog (*Physalaemus pustulosus*) inter-chorus intervals to determine the time period over which females might benefit from retaining attraction to a male's calls (Figure 4.1). Females choose a mate from males calling in a chorus, and assuming that they integrate calls over a substantial time period to assess males, as shown in other anurans (Schwartz et al. 2004), females could reduce assessment time by retaining attraction to a male through the silent intervals between chorus bouts. Reduced assessment time could ameliorate some costs of mate choice, such as increased predation risk characteristic of túngara frog choruses (Ryan et al. 1982; Sullivan 1994). From recordings of chorus calling activity, we determined that chorus bouts (n = 4 chorus groups, 109 bouts) are separated by variable silent intervals with a mean of 25 s (mean +/- SE = 25.2 +/- 1.9 s; Figure 4.2).

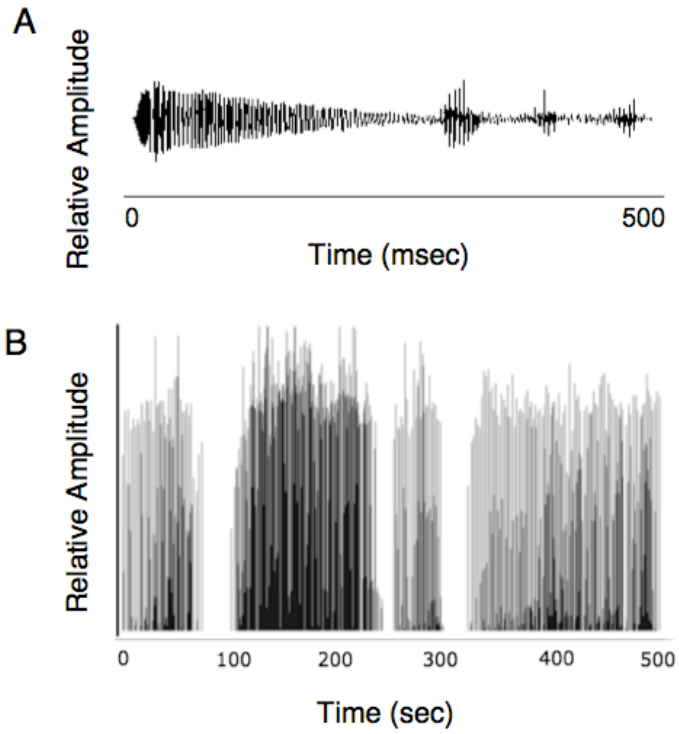

Figure 4.1: Túngara Frog Individual and Chorus Waveforms

Túngara frogs call in choruses that are active in bouts, separated by silent periods. Each male can produce simple and complex calls. (A) A complex call with 3 chucks. (B) A chorus calling in bouts.

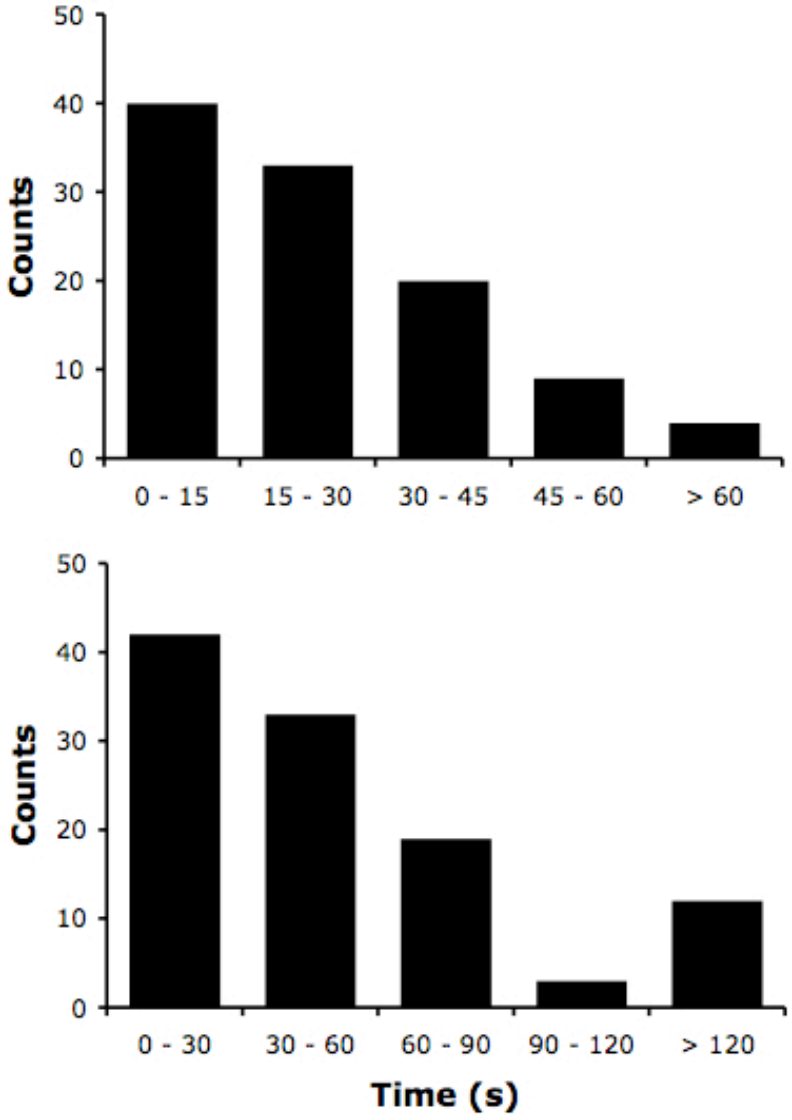

Figure 4.2: Distribution of Natural Chorus Bouts and Intervals

Distribution of inter-chorus intervals (top; N = 106; mean +/- SE = 25.2 +/- 1.9 sec) and chorus durations (bottom; N = 109; mean +/- SE = 55.4 +/- 5.6 sec) of túngara frog choruses over four nights in June 2008 in Gamboa, Panama.

Phonotaxis Experiments

Active Time

We conducted phonotaxis experiments to determine whether complexity, defined here as the number of components in a call (Hebets & Papaj 2005), influenced active time for advertisement signals. Active time is the period over which an ephemeral signal influences a receiver's immediate response to that signal, analogous to a signal's active space, i.e., the area over which it is effective. We restrained a female under an acoustically transparent cone between two speakers. For the first 60 s, two speakers broadcast a series of alternating and identical simple whines. For the next 30 s, one speaker broadcast simple whines while a series of whines with 1 or 3 chucks broadcast from a second speaker. This presentation was followed by a silent period, after which the female was released as both speakers began to broadcast identical simple whines. If females approached the speaker that originally broadcast complex calls, this was evidence that the silent period was within the complex signal's active time. We predicted that active time would be longer for 3-chuck calls than for 1-chuck calls.

One-chuck calls did not influence choices made after a silent period, but 3-chuck calls did so significantly (Figure 4.3). A repeated-measures logistic regression with the general estimating equation (GEE) model showed a significant effect of chuck number on speaker choice for each of the silent periods (N = 240 choices, 133 females; Wald Chi-square = 11.053, df = 1, $p < 0.001$). Pair-wise comparisons showed that the results at each delay period differed significantly between 1-chuck and 3-chuck calls (0 s: $p < 0.009$; 15 s: $p < 0.009$; 30 s: $p < 0.009$).

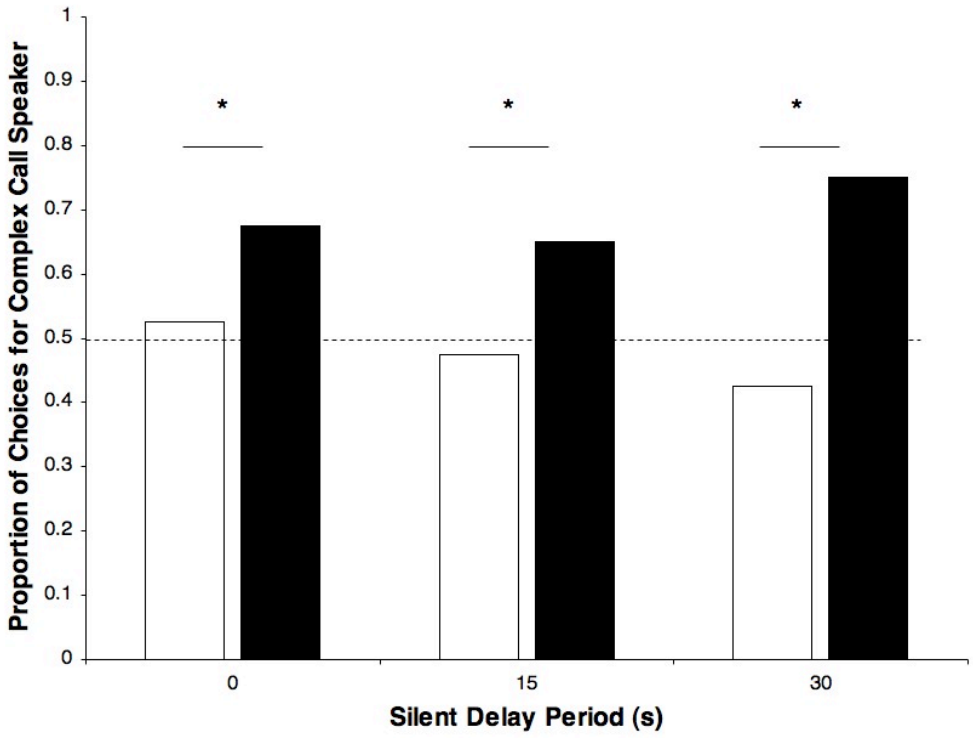

Figure 4.3: Proportion of Choices for Complex Call Speakers

Bars show proportion of choices for the speaker that originally broadcast complex calls before a 0 s, 15 s, or 30 s silent period. Dashed line shows the null hypothesis of 50% choice for each speaker. Females do not discriminate based on prior calls when the initial call pair was whines versus 1-chuck calls (white bars) but do discriminate when the initial call pair was whines versus 3-chuck calls (black bars), preferentially approaching the speaker that broadcast 3-chuck calls (general estimating equation: $n = 240$ choices, $p < 0.001$; pair-wise comparisons with sequential Bonferroni adjustment: * = $p < 0.009$).

We further investigated the active time for 3-chuck calls by testing the temporal limit of this signal's influence on females. Preference for the speaker that broadcast 3-chuck calls was retained after 45 s and reached the null expectation of 50% after 120 s (Figure 4.4). The mean chorus interval in nature is 25.2 s, thus the females retain assessment of 3-chuck but not 1-chuck calls over most inter-bout intervals. Because males call in leks and defend calling territories (Ryan 1985), if a female approaches calls

that come from the same location as earlier attractive calls, she will reach the original signaling male.

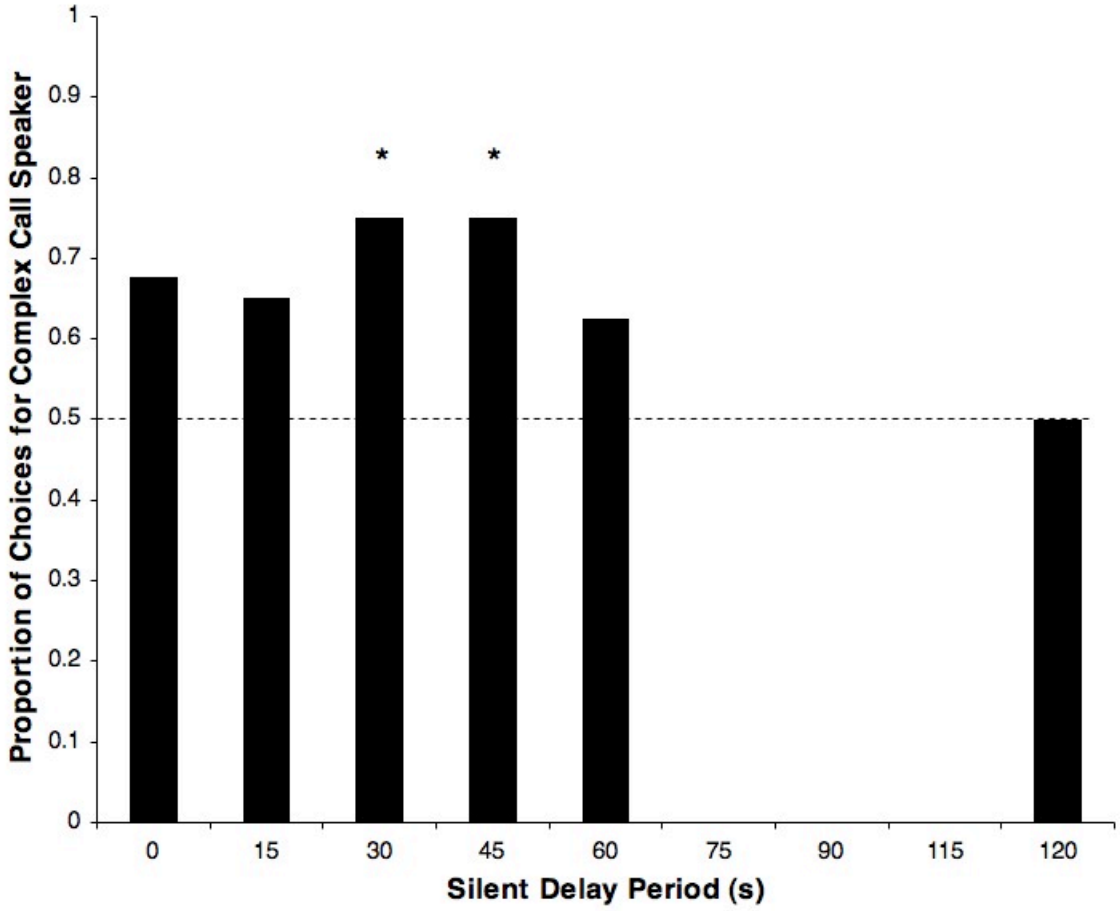

Figure 4.4: Proportion of Choices for 3-chuck Calls Decreases Over Time

Bars show proportion of choices for the speaker that originally broadcast 3-chuck calls before a variable silent period. The preference for complex calls fades after silent periods greater than 45 s (n = 40 each condition, exact binomial with Bonferroni adjustment: * = $p < 0.008$).

Memory, Behavioral Mnemonic, and Motivation

Working memory of an ephemeral signal would extend its active time, but other factors might also influence this measure. For example, maintaining postural orientation

toward a sound after it ceases could be a behavioral mechanism to retain information about an attractive signal's location. We propose the term ''behavioral mnemonic'' to describe this phenomenon of an adaptive strategy to retain information behaviorally without increasing memory load, as in the case of using a bookmark rather than remembering a page number. If females maintain postural orientation toward complex calls through the silent period, then orientation toward the speaker might explain the retention of attraction to complex calls. We recorded female orientation at the moment of release from the cone after the silent period. The GEE logistic model showed no influence of orientation on choice ($n = 231$; Wald Chi-square = 2.447, df = 1, $p = 0.118$), thus females do not use a behavioral mnemonic as an alternative to memory, and this is not a strategy that interacts with memory as the mechanism for retaining attraction to calls.

Increased active time for calls of greater complexity could result from enhanced motivation to approach 3-chuck calls, differential memory of these calls, or an interaction between these factors. We considered several measures of motivation to test the hypothesis that females show differential motivation to approach calls with 1 or 3 chucks.

First, we conducted preference tests between a simple call and either a 1-chuck or 3-chuck call to determine the strength of preference for each type of complex call. In these tests, the female was released as calls were broadcast, and the broadcast continued until a speaker was chosen or the female failed the test by remaining still or not entering the choice zone. The strength of preference for complex calls over simple calls did not differ between 1-chuck and 3-chuck calls ($n = 25$ females; Fisher's exact test: $p = 0.668$).

We also compared latency to make a choice in the two conditions and found no significant difference ($n = 25$; 1-chuck calls: mean +/- SE = 61.0 +/- 14.9 s; 3-chuck calls: mean +/- SE = 74.3 +/- 15.2 s; paired 2-tailed t test: $p = 0.544$). Similarly, chuck

number did not have a significant effect on latency in the active time trials (n = 239 choices; 1-chuck calls: mean +/- SE = 92.3 +/- 8.7 s; 3-chuck calls: mean +/- SE = 99.6 +/- 8.2 s; GEE linear model; Wald Chi-square = 0.406, df = 1, p = 0.524).

We also tested whether females differentially maintain orientation toward complex calls after 3-chuck and 1-chuck calls. The GEE logistic model showed no interaction effect for chuck number and orientation (n = 231; p = 0.810).

Our data reject the hypothesis that differential motivation influences the difference in active time for 1-chuck and 3-chuck calls. Thus, we conclude that increased active time for 3-chuck calls depends upon working memory.

DISCUSSION

Our results show that females retain attraction to ephemeral mating signals over silent periods; when female túngara frogs chose between sources broadcasting identical whines, they preferred the source that previously had been broadcasting 3-chuck calls. Other studies in a variety of taxa have shown that exposure to males during a critical period as a juvenile can influence later preferences as an adult (Hebets 2003) and that recent exposure to males of different attractiveness can influence later female receptivity (Bailey & Zuk 2009; Collins 1995; Wagner et al. 2001; Dukas 2005). Studies of mate choice copying have also shown that recent experience can influence later preference (Schlupp et al. 1994; Galef & White 1998). Our study, however, is the first to show that recent exposure to advertisement signals of certain males influences later female choice between those same males. Our study is also the first to consider the active time of mating signals and to propose its potential role in the evolution of complex mating signals.

We show that active time is enhanced by signal complexity: exposure to 3-chuck calls but not 1-chuck calls is effective after a delay. Thus, female retention of attraction to a caller over silent periods could favor the evolution of call complexity. Complexity in mating signals has been studied extensively, and many hypotheses for its evolution have been considered (Ryan & Keddy-Hector 1992; Searcy & Nowicki 2005; Spencer et al. 2003; Hebets & Papaj 2005; Hartshorne 1973; Searcy 1992; Stripling et al. 1997). In general, hypotheses tend to fall into one of two classes: (1) complexity is an honest signal of male quality or (2) complexity is due to signal efficacy derived from the way signals interact with sensory biases or receiver psychology. Although both factors can interact, our data suggest support for the sensory bias explanation because there are no data to suggest that chuck number indicates better mates (Ryan 1985; Bernal et al. 2007). All male túngara frogs can produce complex calls, and males in a chorus tend to increase chuck number together, in response to other males increasing call complexity (Bernal et al. 2009; Goutte et al. 2010). Nonetheless, it is still possible that females remember complex calls for longer because these calls indicate superior mates.

Memory is the mechanism by which females retain their attraction to complex calls. Female preference strength, latency to choice, and orientation upon release showed no difference between trials with 1-chuck and 3-chuck complex calls. Thus, the difference in active time is not influenced by differential motivation. We have not documented the precise parameters of complexity relevant to differential memory (i.e., duration, total energy), but as more chucks are added, the quantity of sensory stimulation from the advertisement signal increases, as is the case for many instances of complex signaling (Ryan & Keddy-Hector 1992). This difference in stimulation could influence the neural processing of sensory information.

Active time is a metric relevant to a variety of research related to temporal integration, such as mate assessment. For example, one area of research that would have obvious applications for active time is call rate variation (McComb 1991; Galeotti et al. 2005). Faster call rates could ensure that signals with a short active time maintain a continuous influence on female behavior. A related process might explain the similarity between the duration of female túngara frog memory for 3-chuck calls and the duration of silent periods between chorus bouts.

Active time may also help us understand multimodal signaling in animal communication. Additional sensory modalities may increase the active time of a signal, as do additional components within one modality. In túngara frogs, for example, an inflating vocal sac is a visual cue that makes the mating call more attractive (Taylor et al. 2008), and it might also enhance the call's active time. We predict that this active time enhancement might be a general feature of multimodal signals, which may help us understand the occurrence of multimodal signals that have been assumed to be redundant (Partan & Marler 2005).

In summary, this study shows that females retain attraction to ephemeral calls over silent periods and that this effect depends upon the degree of call complexity. Females retain their attraction via working memory. This study highlights the need to consider not only how signal structure influences its attractiveness to the receiver, but also how this influence is retained once the signal ceases and, in general, how cognitive processes interact with preference in decision making. Sexual selection on signals to increase their active time by enhancing receiver memory might explain the evolution of many complex courtship signals. Similar results have documented the influence of long-term memory on warning-signal evolution (Hebets & Papaj 2005; Guilford & Dawkins 1991; Speed 2000). Because working memory also influences human language learning

and comprehension (Baddeley 1992), its influence on nonlanguage signal evolution might provide comparative perspectives on how the brain processes complex signals.

EXPERIMENTAL PROCEDURES

Experiments were conducted in Gamboa, Panama at the Smithsonian Tropical Research Institute (STRI) between June and November of 2007 and 2008. We captured male-female pairs and tested females in a 2.7 x 1.8 m sound attenuation chamber, then released pairs after testing. Chamber temperature was controlled to be 26˚C (range 25˚C–27˚C). Each female performed a block of binary choice phonotaxis tests, and we followed standard toe clipping procedures to mark individuals before release. Toe clip procedures followed the Guidelines for Use of Live Amphibians and Reptiles in Field and Laboratory Research, compiled by the Herpetological Animal Care and Use Committee (HACC) of the American Society of Ichthyologists and Herpetologists, available at http://www.asih. org/files/hacc-final.pdf. All experiments were licensed and approved by STRI, The University of Texas at Austin Institutional Animal Care and Use Committee, and La Autoridad Nacional del Ambiente.

Stimulus Presentation

For each phonotaxis test, we placed a female under an acoustically transparent mesh funnel in the center of the chamber and between two speakers (ADS L200C) placed opposite each other at a distance of 50 cm on either side of the female. The speakers broadcast synthetic calls antiphonally, each at a call rate of one call per 2 s at a peak intensity of 90 dB sound pressure level (re: 20 μPa) at the initial location of the female. Synthetic calls were constructed (software developed by J. Schwartz) to be similar to the population average of male call variation based on seven call parameters (Ryan & Rand 1995).

Phonotaxis Trials

In each trial, we scored female response as a ‘‘choice’’ when she approached to within 10 cm of a speaker within 10 min of being released from the funnel. If she did not reach a speaker within 10 min, failed to leave the center of the chamber within 5 min, or remained immobile for 2 min at any point in the test after leaving the circle, she was scored as ‘‘no choice.’’

We varied which speaker played the complex call, leading call, last call before silence, and leading call after the silent delay. We used both 1- and 3-chuck complex calls and tested females with three silent delay periods, 0 s, 15 s, and 30 s, to compare these two call types. We then titrated the active time of 3-chuck calls by testing females with delay periods of 45 s, 60 s, and 120 s. Females completed a series of tests in random order. Each test had a sample size of 40 females. Most females were tested with several different stimulus combinations on the night of capture, but females were tested only once with any specific stimulus combination. For tests of preference strength, 25 females completed two tests as a repeated measures series.

Statistical Analysis

To determine the influence of chuck number on speaker choice in these tests, we used the GEE procedure in SPSS 16 to create a binary logistic model of speaker choice predicted by the number of chucks in the complex call and the length of silent delay period. Our statistical analysis controlled for the fact that most females completed multiple tests but that few completed all conditions by using the repeated subject function. Pair-wise comparisons were completed with the EMMeans contrasts with sequential Bonferroni adjustments for multiple comparisons.

We also completed GEE models to determine whether chuck number and orientation interacted to predict speaker choice and whether chuck number influenced

latency to choice. The sample sizes for these models differ because orientation was not recorded for 9 of 240 trials and latency was not recorded for 1 of 240 trials.

Natural Chorus Activity

We recorded natural chorus activity to determine the duration of chorus bouts and silent intervals in one standard chorus location. We note that chorus patterns may vary with environmental factors such as frog density, vegetation, and predator density. Here we simply use the data from one location as one example of a relevant chorus pattern. We used an Olympus Digital Voice Recorder WS-100 to record choruses continuously for 8 h, 1830-0230 h, which bracketed most of the activity during four nights in June 2007. We measured the duration of chorus bouts and of inter-chorus intervals using the gating function in Signal®. We analyzed the first five bouts and intervals from each 50 min period between 1830-0120 h, for a total of 109 choruses and 106 inter-chorus intervals.

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Chapter 5: Memorability of variably complex mating signals is non-linear

ABSTRACT

Female choice influences the evolution of male mating signals through sexual selection. Many females prefer more elaborate signals. Female memory for ephemeral mating signals could also influence the evolution of mating signals. In túngara frogs (*Physalaemus pustulosus*) females prefer calls with more chucks to fewer chucks, and have a longer memory for calls with 3 chucks over calls with 1 chuck. Here we examine the relationship between signal complexity (number of chucks) and the females' preference and memory for those signals. Our null expectation was that the two receiver responses should be similar. We found instead that signals elicit memory only at intermediate complexity. We then tested whether this specificity was influenced by (a) chuck number or duration; (b) the whine's and chucks' temporal relationship; or (c) backward masking. Results did not support (a) or (b), but we did find evidence that backward masking might reduce call memorability, and that females exhibit avoidance memory for interrupted series of chucks.

INTRODUCTION

Female choice influences the evolution of male traits through sexual selection (Andersson 1994; Darwin 1871). Frequently, females prefer to mate with males that have exaggerated traits. Sometimes females are even most responsive to supernormal traits, those with trait values exceeding the trait's natural range (Ryan & Keddy-Hector 1992). The relationship between the strength or probability of a female response to a courtship signal and some quantity of the signal can be illustrated in a preference function. In most cases preference functions determined experimentally (Bee 2008; Gerhardt et al. 2000; Kirkpatrick et al. 2006; Ritchie et al. 2001) and those envisioned by

theoreticians (Lande 1981; Cohen 1984; Ryan & Rand 1993; Wagner 1998) are not linear. Even when females show increasing preference for greater trait magnitude the preference often tends to or is predicted to show either a logarithmic response or a peak preference that is above the population average (Figure 5.1).

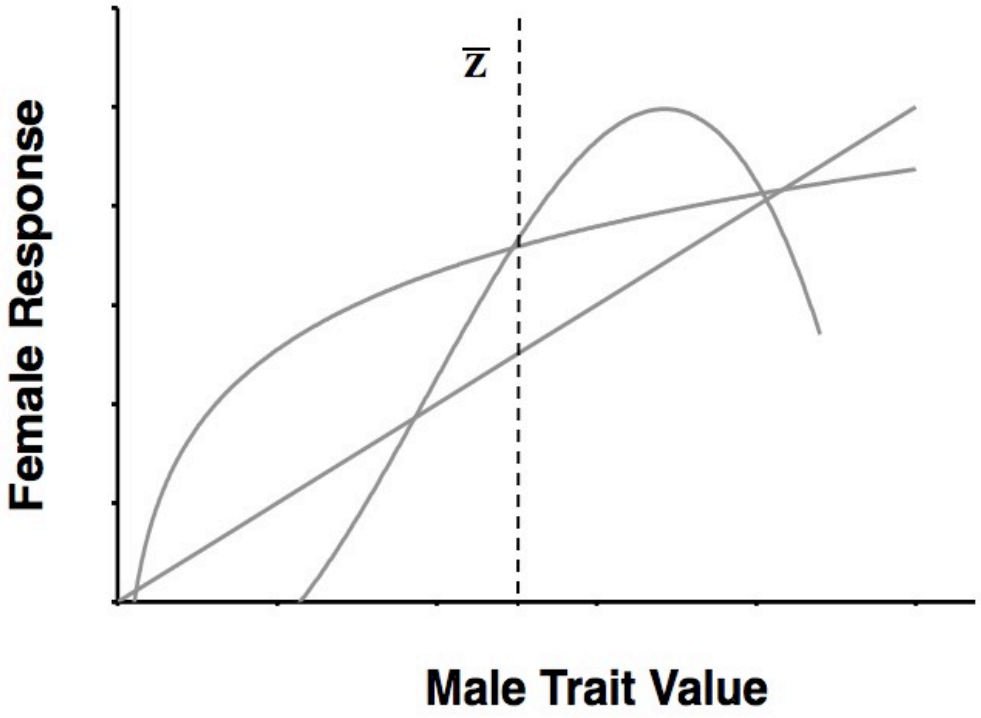

Figure 5.1: Female Response to Variation in Male Traits

Female preference functions can demonstrate linear, logarithmic, and stabilizing shapes. When Z is the average male trait value (dotted line), each of the relationships above places directional pressure on males to evolve more exaggerated traits.

Signal structure influences preference measures such as discrimination and response strength, and it can also influence the signal's memorability (Fetterman 1995; Speed 2000). Memory for communication signals, and especially for mating signals,

could generate strong selection on signal evolution (Ryan et al. 2009). We recently showed that signal complexity influences female túngara frog working memory for male advertisement calls (Akre & Ryan 2010; Chapter 4). Our results demonstrate that an increase in signal complexity can cause increased signal memorability. Here we want to determine the more general relationship between signal complexity and memorability. Female memory might increase linearly with signal complexity, but the relationship between female memory and signal variation can take many forms, as occurs in preference functions. In túngara frogs, discrimination between signals of variable complexity demonstrates that females prefer greater complexity, but are most responsive to calls of intermediate complexity, because discrimination appears to be constrained by Weber's law (Akre et al., in prep, Chapter 3). Psychophysical models of stimulus perception, such as Steven's power law, are similar in form to models of stimulus forgetting (Fetterman 1996), but this does not guarantee that preference and memory exhibit the same relationship to signal variation. For example, several studies of memory for acoustic stimuli demonstrate the "choose-short effect," such that response accuracy decays faster for longer duration stimuli relative to short ones (Fetterman 1995).

Túngara frog advertisement calls can have two parts: an initial low frequency sweep called a "whine," and a terminal high frequency burst called a "chuck" (Ryan 1985; Ryan & Rand 2003a). Whines may be voiced alone (simple calls), or followed by 1 to 7 chucks (complex calls). We refer to complexity as the number of components in the call; thus calls with more chucks are more complex. Males call in choruses, and within a calling bout males usually produce both simple and complex calls of variable chuck number (Bernal et al. 2007). Chorusing occurs in bouts separated by periods of silence that last an average of 25 s (Akre & Ryan 2010). Females exhibit a strong preference for complex to simple calls [for 1-chuck: 86%, N = 3662 (Gridi-Papp et al.

2006)]. Females do not consistently prefer greater complexity, or more to fewer chucks (Bernal et al. 2009), but at close proximity, around 0.5 m, they do exhibit consistent preference for greater complexity, although the strength of this preference diminishes as stimulus magnitude increases, according to the predictions of Weber's law (Akre et al. in prep). Memory, too, is proximity-dependent, since females remember 3-chuck but not 1-chuck calls at high amplitude (Akre & Ryan 2010), but memory does not occur at lower amplitude (Akre & Ryan in prep).

We tested female memory for several synthetic acoustic stimuli to determine how mating signals influence female memory response. Frogs have two inner ear organs with different frequency ranges to which they are most sensitive: the amphibian papilla (AP) is most sensitive to frequencies below 1200 Hz while the basilar papilla (BP) is most sensitive to frequencies above 1500 Hz. The AP has lower response thresholds than the BP (Capranica 1976; Ryan et al. 1990). The addition of chucks to túngara frog calls adds energy in a higher frequency range to which the BP is most sensitive: about 90% of a chuck's energy is in frequencies > 1500 Hz (Ryan et al. 1990). Although females respond specifically to chucks at least as far as 3 m from a male (M. Ryan & S. Rand, unpubl. data), the ability to discriminate between chuck numbers and to remember chuck production appears to require greater BP stimulation. Thus we predicted that because increasing chuck number beyond 3 would increase the energy stimulating the BP, this would also increase the previously established memory response to 3-chuck calls. We first tested whether females increased memory with increasing call complexity. We predicted 3 chucks to be more memorable than 2, but we predicted 4 chucks to be more memorable than 3, and 5 to be more memorable than 4 chucks.

When results did not support our predictions, we designed further experiments to understand the difference in female response to 3 and 4 chucks. We investigated the

influence of the number and duration of components and a variety of temporal relationships between signal components on signal memorability. These factors influence working memory for a variety of acoustic stimuli in humans and some other primates (Acheson & MacDonald 2009; Brosch et al. 1998; Fetterman 1995), but they have not been applied to studies of acoustic communication signals in other taxa. Each test gives females a delayed choice between a simple whine and a complex call—that is, a whine followed by some combination of chucks.

GENERAL METHODS

Data were collected in Gamboa, Panama, at the Smithsonian Tropical Research Institute (STRI) between June and September of 2008 and 2009. Male-female pairs in amplexus were brought to our laboratory for testing, and then released after testing the same night at the site where they were originally collected. We stored pairs in the laboratory in dry dark containers to reduce stress and avoid moisture that could stimulate the female to drop her eggs. To avoid using the same female more than once in a given test, we followed standard toe-clipping procedures to mark individuals before release. Toe clip procedures followed the Guidelines for Use of Live Amphibians and Reptiles in Field and Laboratory Research, compiled by the Herpetological Animal Care and Use Committee (HACC) of the American Society of Ichthyologists and Herpetologists, available at: http://www.asih.org/files/hacc-final.pdf. All experiments were licensed and approved by STRI, The University of Texas at Austin IACUC, and La Autoridad Nacional del Ambiente of the Republic of Panamá.

We used female phonotaxis in a binary choice test as an assay for call preference and memory. We followed previously successful procedures with some modifications (Akre & Ryan 2010; Ryan 1985; Ryan & Rand 2003a). For each test, we placed a female

under a mesh cone in the center of a sound attenuation chamber measuring 2.7 x 1.8 m, between two speakers (ADS L200C) placed at a distance of 50 cm on either side of the female. Testing was monitored with an IR illuminated camera (Fuhrman Diversified, Inc.) projecting an image of the chamber floor to a video screen outside the chamber. The speakers broadcast antiphonal calls every 1 sec such that peak intensity level of the whine at the center of the chamber was 90 dB SPL (re. 20 μPascals). The initial stimulus pair was identical whines. After 60 sec, one speaker continued to broadcast the whine while the other speaker broadcast a whine plus some combination of chucks; both speakers broadcast their calls antiphonally for 30 sec. A silent delay followed this presentation, and then we lifted the cone as speakers broadcast identical whines again until the female chose a speaker or failed the test. Once a female was released, we scored her response as a choice for one of the speakers when she approached to at least 10 cm of that speaker within 10 min of being released from the cone. If she did not reach a speaker within 10 min, if she failed to leave the center of the chamber within 5 min, or if she remained immobile for 2 min at any point in the test after leaving the circle, she was scored as "no choice."

The Relationship between Chuck Number and Memorability

We tested whether female memory for complex calls increases as the number of chucks in the signal increase. We previously tested memory for 1 and 3 chucks over a silent delay and found that females exhibited a preference for the speaker that had previously broadcast the 3-chuck call, but did not exhibit an analogous preference for the 1-chuck call. We interpreted these results as evidence for female memory for calls with 3 chucks but not with 1 chuck (Akre & Ryan 2010). In the current study, we further

explored this relationship by testing whether females remember 2, 4, and 5-chuck calls after the silent period.

Methods

As in the previous study, stimuli were synthetic calls that were created to match the average call characteristics of the population (software developed by J Schwartz). We tested 2, 4, and 5-chuck calls over a 30 s delay period, then repeated this test with 2, 3, and 4 chucks over a 45 s delay, and 2, 3, and 4 chucks over a 60 s delay. For each silent delay period, we tested 40 females in a repeated measures design where females completed all tests in random order. We used a one-tail exact binomial test because we specifically predicted that greater memory effects would be elicited by more complex calls (call with chucks) compared to the simple call (the whine with no chucks). We then used a Fisher's exact test (two-tailed) to compare the strength of the response between pairs of experiments that differed in chuck number. Data for 3-chuck calls were previously published in a separate analysis (Akre & Ryan 2010).

Results

Females did not exhibit memory for 2, 4, or 5 chucks after a 30 s delay (one-tailed exact binomial, N = 40 females, 2 chucks: $p = 0.3179$; 4 chucks: $p = 0.5627$; 5 chucks: $p = 0.4373$; Figure 5.2a). Comparing these results to data on response to 3-chucks after a 30 s delay (Akre & Ryan 2010) shows that 3-chuck calls are not different from 2-chuck calls ($p = 0.1000$) but are different from 4-chuck calls ($p = 0.0368$) after the same delay. After a 45 s delay, females remember 3 but not 2 or 4-chuck calls (N = 41 females, 2 chucks: $p = 0.3776$; 3 chucks: $p = 0.0022$; 4 chucks: $p = 0.5$; Figure 5.2b). The response to 2 chucks is not significantly different from the response to 3 chucks after a 45 s delay ($p = 0.1078$), but the responses to 4 and 3 chucks are different ($p = 0.0408$). After a 60 s

delay, females do not remember 2, 3, or 4-chuck calls (N = 40 females, 2 chucks: p = 0.5627; 3 chucks: p = 0.0770; 4 chucks: p = 0.4373; Figure 5.2c). Neither 2 nor 4 chucks are significantly different from 3 chucks after a 60 s delay (2 versus 3: p = 0.3675; 3 versus 4: p = 0.2611).

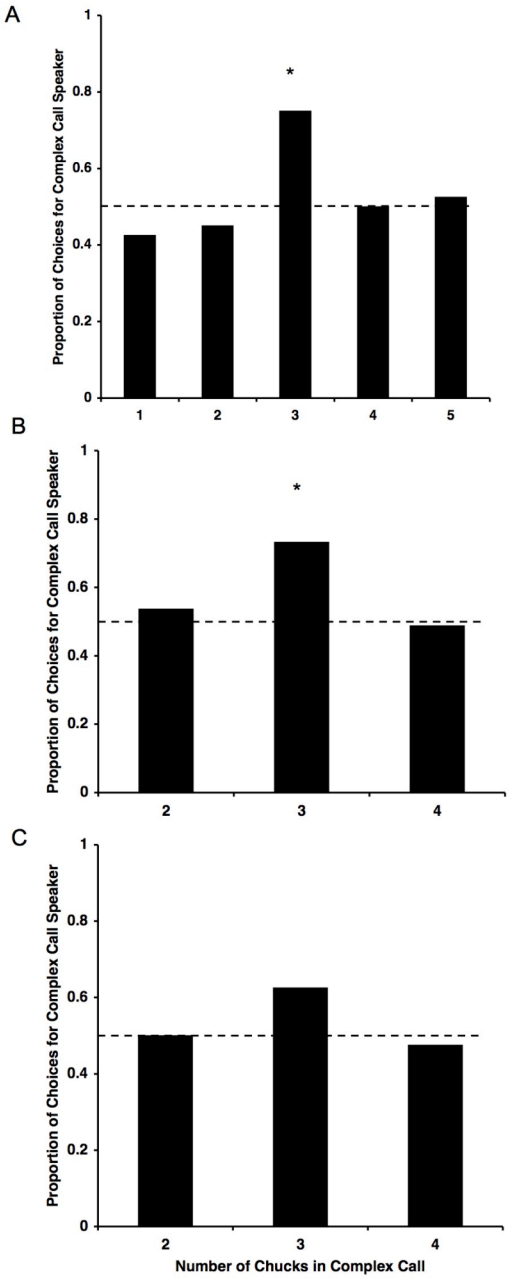

Figure 5.2: Female Memory for Signals of Variable Chuck Numbers

Bars show the proportion of females that chose a speaker that broadcast complex calls before a silent delay of A) 30 s, B) 45 s, and C) 60 s (N = 40, * = $p < 0.05$). The dashed line represents the null hypothesis.

Discussion

As we predicted, 2 chucks were less memorable than 3 chucks. Counter to our predictions, however, neither 4 nor 5 chucks were more memorable than 3 chucks, thus female memory does not consistently increase with stimulus magnitude. Female memory for 3-chuck calls differs significantly from memory for 4-chuck calls after 30 s and 45 s of silence. Though memory for 3-chuck calls is not significantly different from 2-chuck calls in either condition, 3-chuck calls tend to be more memorable than 2-chuck calls in all time periods tested. This was not surprising because signal properties that enhance salience are known to increase memorability (Speed 2000). The difference between 4-chuck and 3-chuck calls was more surprising, and we pursued this difference in the remainder of the study.

Exploring Peak Memorability at Intermediate Complexity

We asked why more chucks are not always more memorable, focusing on the difference between 3 and 4 chucks. Three and 4-chuck calls differ in number of components, total call duration, and the temporal relationship between the chucks and the whine. We designed tests to address whether these features were responsible for the lack of memory for 4-chuck calls. For all remaining tests, the silent delay period is 30 s.

Number and Duration

Many taxa exhibit sensitivity to number of objects (Hauser et al. 2000; Pisa & Agrillo 2009), and some animals appear to remember number for long enough to make decisions based on mental arithmetic (Rugani et al. 2009). Controlling for a variety of cues such as duration of acoustic stimuli or area of visual stimuli, however, can extinguish the influence of number on behavior (Pisa & Agrillo 2009; Santi & Van Rooyen 2007), suggesting that animals can use a summation of event stimulation rather

than counting to discriminate between numbers. We tested whether 3-chuck calls are more memorable than 4-chuck calls due to duration of the total chuck-series in each, or the number of discrete components.

Methods

We created the following stimuli: "cccc" : a whine followed by 4 short chucks whose total duration is equal to the total duration of 3 normal chucks; and, "**CCC**" : a whine followed by 3 long chucks whose total duration is equal to the total duration of 4 normal chucks (Figure 5.3). If total chuck-series duration is responsible for the memorability of 3-chuck calls, females would remember cccc but not **CCC**. If the number of discrete components is responsible, females would remember **CCC** but not cccc.

Results

Females did not exhibit memory for cccc (one-tailed exact binomial, $N = 37$, $p = 0.500$) or **CCC** ($N = 38$, $p = 0.436$).

Discussion

Results suggest that chuck number alone is not sufficient to instantiate memory, nor is summed chuck duration. These surprising results indicate that the interaction between these two features is relevant to memorability. The natural relationship between chuck duration and number, however, has not been explored. Males may be constrained in chuck duration as they increase chuck number.

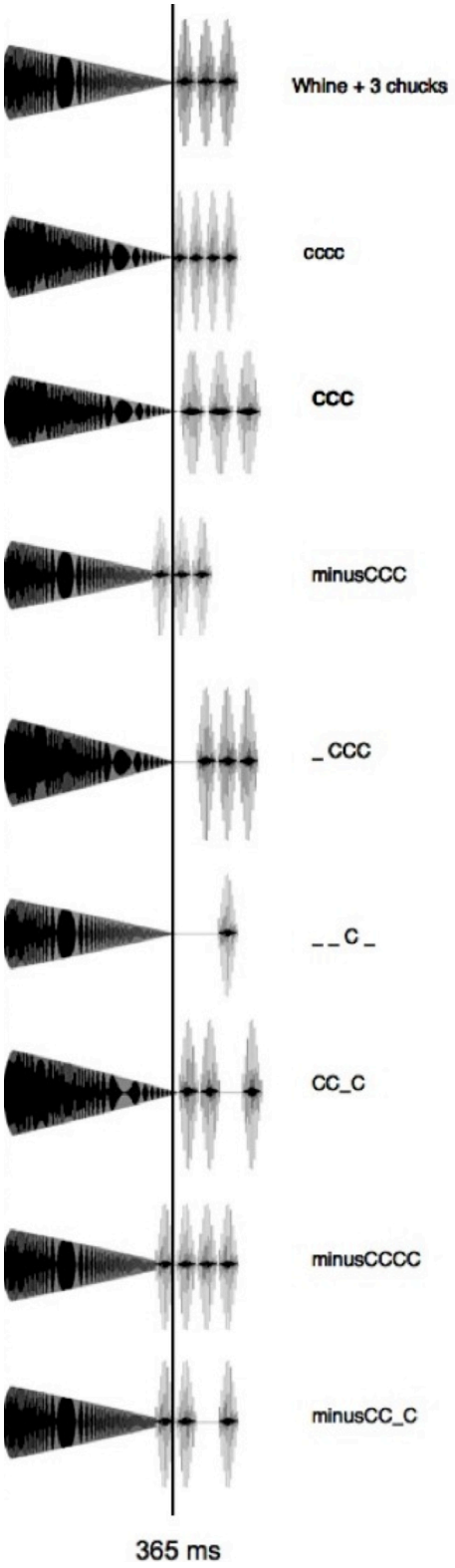

Figure 5.3: Waveforms of Stimuli

Each waveform is labeled with the name used in the body of the paper. The line bisecting all stimuli shows the end of the whine, which is 365 ms in duration for all stimuli. The y-axis measures amplitude. The whine to chuck relative amplitude was equal for all stimuli.

Temporal Relationship Between Whine and Chucks

Neural processing of acoustic stimuli is time-sensitive in many anurans (Alder & Rose 1998; Gerhardt & Huber 2002; Gerhardt & Schul 1999), and the study by Wilczynski et al. (1999) shows this is true for túngara frogs as well. Human studies show that the processing of different signal features occurs with variable timing, such that temporal integration of signal features requires brief storage of certain feature responses prior to summation, during which time they may be amplified or attenuated (Naatanen & Winkler 1999). We tested whether the temporal relationship between the whine and chucks influences memory, as it does female preference (Wilczynski et al. 1999). In tests of preference, the chuck's influence on the call's attractiveness is greatest in its normal position, but there is an otherwise fairly permissive relationship between whine and chuck position; even when a chuck is placed before the whine it enhances the call's attractiveness. We wanted to know if the memory effect of 3-chuck calls is dependent on the chuck series' precise temporal relationship to the whine.

Methods

We manipulated the 3-chuck call either by moving the 3-chuck series 45 ms (the duration of one chuck) towards the whine, such that it overlaps the whine, or away from the whine, such that it leaves a silent interval between the whine and chuck. In this way we created the following stimuli: "minusCCC" : a whine followed by 3 chucks that have been shifted 45 ms to overlap with the final portion of the whine; "_CCC" : a whine followed by 3 chucks that have been shifted to initiate 45 ms later than the normal position of the chuck series relative to the whine (Figure 5.3). If temporal relationship with the whine does not influence memory, females should remember both minusCCC and _CCC.

We also wanted to know whether hearing a single chuck in the position typically occupied by the 3rd chuck in a 3-chuck series was by itself sufficient for memory, and if it was necessary for memory of 3 chucks. To test this we created the following stimuli: "_ _C" : a whine followed by 1 chuck shifted later temporally to fill the position normally held by the 3rd chuck in a series; and "CC_C" : a whine followed by 3 chucks in the positions normally filled by chucks 1, 2, and 4 (Figure 5.3). If a chuck in the 3rd chuck position is sufficient to stimulate memory, females should remember _ _C, and if it is necessary they should not remember CC_C.

Results

Females remember the 3-chuck calls when the chuck series was shifted earlier (minusCCC: N = 40, p = 0.008), but not when it was shifted later (_CCC: N = 36, p = 0.434). A single chuck in the position normally occupied by chuck number 3 does not elicit a memory effect from the females (_ _C: N = 40, p = 0.437). Females did exhibit memory for an interrupted series of 3 chucks, but unexpectedly the females avoided the speaker that previously broadcast this call (CC_C: N = 40, p = 0.040).

Discussion

A chuck in the typical 3rd chuck position is neither necessary nor sufficient for the 3-chuck call to elicit a memory effect. Both the tests of minusCCC and CC_C showed a memory effect, and neither have a chuck in the typical 3rd chuck position. Tests of both _ _C_ and _CCC failed to show a memory effect, and both have a chuck in the typical 3rd position. Thus differential memory response is not explained by a unique property of the 3rd chuck position relative to the whine.

Backward Masking

The temporal relationship between individual chucks might also influence memory response. Backward masking is a process by which the presence of a later stimulus component that immediately follows an early stimulus component can interfere with late-response neuronal activation, such that the later stimulus effectively masks the earlier one (Brosch et al. 1998). This phenomenon occurs at the level of sensory perception, prior to cognitive processes such as short-term memory (Rammsayer & Lima 1991). Although best explored in primate psychophysics, backward masking is known to occur in birds (Dooling & Searcy 1980) and goldfish (Popper & Clarke 1979), and these studies mention the probability that this influences the evolution of communication signals. We tested the hypothesis that the 4th chuck could extinguish the memory response to calls with a 3-chuck series by backward masking.

Methods

We created the following stimulus: "minusCCCC" : a whine followed by 4 chucks that have been shifted 45 ms earlier to overlap with the final portion of the whine (Figure 5.3). This placed the first chuck overlapping the whine and the 4th chuck in the temporal position of a typical 3rd chuck. The stimulus minusCCC did instantiate memory in females. If a 4th chuck masks the 3rd chuck in a series, females would not remember minusCCCC.

Results

Females did not exhibit memory for minusCCCC ($N = 36$, $p = 0.4340$), therefore backward masking might occur.

Discussion

Adding a 4th chuck to the shifted 3-chuck stimulus extinguished memory, as predicted if backward masking influences signal memory. We cannot be certain that the lack of memory in this test results from backward masking, but this process does occur at a time scale relevant to these stimuli—most stimuli that mask preceding stimuli occur less than 50 ms later (Rammsayer & Lima 1991; Yost 1994), and anurans are known to integrate information over similar time scales (Alder & Rose 1998). This temporal pattern would allow for the 4th chuck to mask the 3rd, but the reason that the 3rd would be critically important is still not clear.

Another possibility (that was not tested) is that the 4th chuck masks not the 3rd chuck, but the increased response that occurs at the transition from whines to chucks. Mismatch negativity is a strong neural response that occurs when there is a change in auditory stimuli, such as a change in frequency. The response coding this change could be backward masked by a following stimulus if it occurs at the peak neuronal responsiveness to frequency change, which is estimated to occur between 150 and 250 ms later in humans (Naatanen et al. 2007). The 4th chuck begins just short of 150 ms after the transition from whines to chucks occurs. Thus, the 4th chuck could mask the benefit of a 3rd chuck, or it could mask the sudden stimulation of the BP that occurs when chucks begin.

Avoidance of Interrupted Series

Having a chuck in all positions but the 3rd (CC_C) elicited an avoidance memory response. We wanted to know whether this effect was due to (a) the interruption of a chuck series having a negative influence on female attraction, or (b) the lack of a chuck in position 3 decreasing attraction and the presence of a chuck in position 4 increasing aversion. To test this we shifted this CC_C series such that it overlapped with the whine,

leaving the gap in position 2 and the final chuck in position 3. We predicted that this series might re-capture the positive memory response.

Methods

We created the following stimulus: "minusCC_C" : a whine followed by 3 chucks in an interrupted series identical to CC_C but shifted 45 ms earlier to overlap with the terminal portion of the whine (Figure 5.3). If an interrupted series instantiates avoidance memory, females should respond to minusCC_C with avoidance memory as they did in response to CC_C. If the 4th position was responsible for the avoidance memory, then females should not show avoidance memory to minusCC_C.

Results

Females showed avoidance memory for minusCC_C (N = 40, p = 0.0403; Results for all experiments summarized in Table 1).

Table 5.1: Female Memory Response After a 30 s Delay

Stimulus	Attraction	Avoidance	No memory
Whine + 2 chucks			X
Whine + 3 chucks	X		
Whine + 4 chucks			X
Whine + 5 Chucks			X
Cccc			X
CCC			X
minusCCC	X		
_CCC			X
_ _ C _			X
CC_C		X	
minusCCCC			X
minusCC_C		X	

Discussion

The stimulus minus CC_C elicited avoidance memory, thus we concluded that gaps within chucks are aversive to females, perhaps because it is an unnatural stimulus. An alternative explanation for avoiding interrupted chuck series is that females are temporally integrating the post-gap chuck to the alternate whine in a way that instantiates attracted memory to that stimulus. Túngara frogs exhibit increased attraction to whines that have a chuck affixed after the whine, in the natural position, but also prior to the whine (Farris et al. 2005; Wilczynski et al. 1999). A significant increase in attractiveness occurs when the chuck begins even 600 ms prior to the start of a whine (Wilczynski et al. 1999). In addition, túngara frogs do not use spatial location to integrate sounds that occur in a sequential fashion (Farris et al. 2002), instead frogs respond to spatially dispersed whines and chucks by approaching the chuck significantly up to a 135˚ separation, but for a 180˚ separation, frogs approach the whine or the chuck in equal proportions. Our study used an inter-call interval such that each speaker broadcast calls every 2 s in alternating fashion. The duration of the whine is 365 ms, and each chuck is about 45 ms separated by 4 ms of silence, thus the post-gap chuck would occur less than 600 ms prior to the alternate whine for both minusCC_C and CC_C. A chuck placed at this temporal relationship with a whine significantly increases the whine's attractiveness (Wilczynski et al. 1999).

Our tests included other cases of interrupted stimuli: _ _C_ and _ CCC, but these were not interrupted in the series of chucks. These were interrupted between the chucks and the whine. These interruptions did not cause avoidance memory, but they may have caused confusion about which whine to integrate with the chuck(s), leading to a 50/50 choice for each speaker – which was the result in this study. This was also the result

when Farris et al. (2005) tested response to speakers that broadcast chucks (a) temporally located prior to the whine and (b) spatially separated from the whine, when speakers were 180˚ apart. Future experiments conducted with an increased inter-call interval can test whether temporal integration confuses female memory.

GENERAL DISCUSSION

The patterns of female delayed response to complex mating stimuli could result from two general explanations. (i) A general psychoacoustic phenomenon influences females such that they remember some but not other stimuli, as is true in many taxa in variable contexts (e.g. predator memory, artificial lab tasks, human memory), or (ii) animals remember all stimuli but choose not to respond to some stimuli due to reasons specific to túngara frogs. The difficulty distinguishing between these two interpretations is similar to problems with tests of signal discrimination, whereby the receiver may respond according to ability to discriminate, or just noticeable differences, or relevance of the difference between stimuli, or just meaningful differences (Nelson & Marler 1990; Ryan et al. 2007; Shettleworth 1998). The most parsimonious explanation would be that females selectively remember according to acoustic stimulus properties, but we acknowledge that both classes are possibilities.

Female túngara frogs exhibit memory for the previous location of a complex call after silent delays of up to 45 sec. Akre and Ryan (2010) showed that this memory effect is elicited by calls with 3 chucks but not calls with 1 chuck. Those results suggested that increasing complexity enhances memory for the signal, and that the relationship between memorability and chuck number could be linear, logarithmic or exponential. Our results here show this is clearly not the case, as females appear to respond to 3-chucks in a unique way. The difference between memory for a 3-chuck and a 4-chuck call is not

explained by a difference in chuck number, chuck duration, or the temporal relationship between whines and chucks. The data are consistent with the hypothesis that a 4th chuck might extinguish memory for a 3rd chuck through backward masking. Data also show that interrupted series of chucks cause avoidance memory.

The relationship between female memory for attractive calls and chuck number is similar to patterns of female preference for greater chuck number, but not identical. Females prefer 3 to 1 chucks, and they remember 3 chucks, but not 1 chuck. However, females prefer 4 to 2 chucks, and they do not remember either 4 or 2 chucks. Response patterns in discrimination tests suggest that female preference for greater chuck number is constrained by Weber's law, a law of psychophysics (Akre & Ryan in prep). Research in psychophysics relates true and perceived stimulus value, and shows that the two do not scale to one another in a linear fashion. Discrimination and memory might be linked simply because they both require analysis of perceived stimuli, so factors that influence perception will influence both discrimination and memory. For example, both subjective sensation of stimulus magnitude and forgetting stimulus features over time follow a power law (Wixted & Ebbesen 1991). Since females show a peak preference for 3-chuck calls when discrimination between 1-chuck calls and calls of all other chuck numbers (0, 2, 3, 4, and 5 chucks; Akre et al. in prep), a peak memorability for 3 chucks is not as surprising as would be peak memory for 2 or 4 chucks.

An alternative explanation is that females integrate late-occurring chucks with the wrong whine. Since a significant increase in attractiveness occurs by adding a chuck prior to the start of a whine, even 600 ms prior to the whine's start (Wilczynski et al. 1999), túngara frogs may be confused by the 4th and 5th chucks in the chuck series tested in the initial portion of this study. Although females did not exhibit avoidance memory in those tests, if some frogs integrated the final chucks with the alternate whine, the result

might suggest no discrimination. As mentioned before, we used an inter-call interval such that each speaker broadcast calls every 2 s in alternating fashion. The duration of the whine is 365 ms, and each chuck is about 45 ms separated by 4 ms of silence, so the duration of a 3-chuck call is about 512 ms, a 4-chuck call is about 561 ms, and a 5-chuck call is about 610 ms in duration. Adding a 4th chuck might decrease the inter-call silent period enough to introduce confusion into which whine is linked to the chuck series in a way that prevents memory from maintaining an association with the correct whine. Also, the separation between chucks in the stimuli tested in the interrupted series avoidance tests might especially confuse females that must integrate chucks with a whine, and this might have influenced the significant avoidance result in those tests.

If females do have trouble integrating the 4th chuck with the correct whine, then frogs in natural choruses would also be susceptible to this problem. Males may face a problem of spacing their calls relative to other males such that females are not confused about which chuck came from which male. This leads to a prediction that males calling alone would use faster call rates, while males calling with other males would prefer to call long after previous calls when adding chucks, or soon after other calls when producing fewer chucks. Generally, males of other species of frogs have been found to lower their rate of calling in larger choruses (Schwartz et al. 2001). Another concern with applying this carefully controlled data on memory with the natural chorus activity is that chorus noise might influence memory as it does discrimination, by reducing female responsiveness to differences between stimuli, which reduces the effectiveness of longer calls in particular (Bee 2008; Schwartz et al. 2001).

Males rarely produce calls with many chucks in natural choruses. Both 3 and 4 chucks are quite rare in natural choruses or single male call bouts, accounting for less than 1% of all calls produced (Bernal et al. 2007). Chuck production, however, is

context-dependent, and males increase chucks in response to elicitation movements by females (Akre & Ryan in prep), but also in general when females are present. When females are present, production of 3-chuck calls in particular increases, accounting for about 11% of calls, while 4-chuck calls account for less than 2% of all calls when females are present (Akre & Ryan, unpubl. data). The rarity of producing 4-chuck calls may relate to the lack of female memory for these calls, which decreases the benefit for males, as well as costs or constraints in producing them.

The nature of acoustic stimuli, as natural communication signals or controlled artificial sounds, probably has a major influence on how animals process signals (Medin & Dewey 1986). That is, response to non-functional stimuli may not elicit the same neural recruitment that arises when stimuli are recognized as relevant to behavioral decisions. Human studies have shown that the same stimuli are processed differently when used in linguistic and nonlinguistic tasks (Wood et al. 1971). In birds, tonal stimulus memory can be surprisingly short relative to the duration of male advertisement vocalizations (Zokoll et al. 2008). Testing response to natural signals may more closely reflect natural processes. Overall, stimuli modified in unnatural ways had little influence on female response in our study.

In summary, female memory for complex calls does not consistently increase with complexity. Backward masking might influence female signal memory. Constraints on temporal integration during short inter-call intervals might explain the memory patterns revealed in this study. Alternatively, peak memorability for 3-chuck calls might result from the same processes that influence preference patterns among multi-chuck calls. Females appear to show a special response to 3-chuck calls—to humans seven might be somewhat magical (Miller 1956) but with túngara frogs three carries more of a spell.

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Vita

Karin Lise Akre grew up in Duluth, MN. She graduated from Harvard University in 1999 with a B.A. in Psychology and Biology. There, she completed an undergraduate thesis project on hemispheric asymmetry of facial expressions and vocalizations in rhesus macaques. She then worked in travel writing, environmental consulting, and science textbook editing before deciding to pursue a graduate degree in Ecology, Evolution and Behavior at The University of Texas at Austin. Her graduate studies included research on communication and cognition in *Physalaemus pustulosus*, the túngara frog, at the Smithsonian Tropical Research Institute in Panama, and in *Lolliguncula brevis*, the brief squid, at The University of Texas Marine Science Institute in Port Aransas, TX.

Permanent address (or email): akre@post.harvard.edu

This dissertation was typed by the author.